

The University of Chicago

STUDIES ON SOYBEAN PROTEIN

I. PEPTIZATION OF SOYBEAN PROTEIN

The Effect of Neutral Salts, and Acids and Bases with and without Added Salts, on the Quantity of Nitrogenous Constituents Extracted from Oil-Free Soybean Meal

II. PRECIPITATION OF SOYBEAN PROTEIN

Precipitation from Water and Alkaline Dispersions of Oil-Free Soybean Meal by Acids and by Electrodialysis

III. THE EFFECT OF FORMALDEHYDE ON THE ISO-ELECTRIC POINTS OF SOYBEAN AND OTHER PROTEINS BY MICROELECTROPHORESIS

A DISSERTATION SUBMITTED TO THE FACULTY OF THE
DIVISION OF THE PHYSICAL SCIENCES IN CANDIDACY
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY
1941

By
SIDNEY J. CIRCLE

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"Peptization of Soybean Proteins. Extraction of Nitrogenous Constituents from Oil-Free Meal by Acids and Bases with and without Added Salts." Ind. Eng. Chem., 30, 1414-18 (1938).

"Soybean Protein. Precipitation from Water and Alkaline Dispersions by Acids and by Electrodialysis." Ind. Eng. Chem., 31, 1284-88 (1939).

"Soybean Protein. Résumé and Bibliography." U. S. Department of Agriculture, Bureau of Agricultural Chemistry and Engineering, 1940.

"The Effect of Formaldehyde on the Isoelectric Points of Some Proteins by Microelectrophoresis." J. Phys. Chem., 45, In Press (1941).

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FOREWORD

This study of soybean protein has been divided for the sake of convenience into three sections, under the separate headings:

- I. PEPTIZATION OF SOYBEAN PROTEIN.
- II. PRECIPITATION OF SOYBEAN PROTEIN.
- III. THE EFFECT OF FORMALDEHYDE ON THE ISOELECTRIC POINTS OF SOYBEAN AND OTHER PROTEINS BY MICROELECTROPHORESIS.

PART I

PEPTIZATION OF SOYBEAN PROTEIN

Introduction

The classification of proteins^{1,2} proposed in 1908 was based mainly on their physical properties, especially solubility and precipitability, at least for the so-called simple proteins. It has persisted to the present time with but slight modifications. Although this classification undoubtedly has been of great value pending a more precise one, there has been a tendency to overlook its inadequacies.

The newer physical methods for the study of proteins permit a more precise characterization of them as chemical entities in certain cases. Thus where a protein may be crystallized, maintains a constant composition after repeated crystallizations, and is monodisperse as shown by sedimentation rates in the ultracentrifuge and mobilities in the electrophoresis apparatus, it may be concluded that it exists as a chemical individual, but even here it must be noted that most crystalline proteins contain small amounts of water and salt or other substances from the mother

¹Joint Recommendations of the Physiological and Biological Committees on Protein Nomenclature, J. Biol. Chem., 4, xlviii (1908).

²R. A. Gortner, "Outlines of Biochemistry," John Wiley & Sons, New York, Second Edition, 1938. This book contains excellent summaries of protein and colloid chemistry.

liquor.

For the vast majority of proteins, the validity of the definitions in the classification is questionable. Especially is this so in the case of the globulins, which are defined as simple proteins, insoluble in water, but soluble in dilute neutral solutions of the salts of strong bases and acids.

The first serious criticism of the classification came from Gortner and his associates.^{3,4} In working on a wide variety of seeds, they found that the amount of nitrogenous matter extracted depended on the kind and concentration of salt used. They concluded that a definition based upon solubility, i.e., peptization, in a dilute salt solution was too ambiguous to have meaning.³ Saunders and his associates,⁵⁻⁷ from their studies on the globulins of several seeds, reached a similar conclusion. A more extreme point of view is found in a study of the salt peptization of wheat proteins by Rich,⁸ who concludes that there is but one protein originally present, the peptizing agents breaking off different parts of the micelle.

A review of the literature disclosed that no systematic study on the salt peptization of soybean proteins has been made. The few published researches touching on this subject have been fragmentary and primarily intended to separate and identify the proteins according to the 1908 classification. Most workers⁹⁻¹¹ in this field followed to a great extent the lines laid down by Osborne and Campbell,¹² who identified the principal protein in

³Gortner, Hoffman, and Sinclair, Colloid Symposium Monograph, 5, 179 (1928).

⁴Staker and Gortner, J. Phys. Chem., 35, 1565 (1931).

⁵O'Hara and Saunders, J. Am. Chem. Soc., 59, 352 (1937).

⁶Saunders, ibid., 53, 696 (1931).

⁷Rotha and Saunders, ibid., 54, 342 (1932).

⁸Rich, Cereal Chem., 13, 522 (1936).

⁹Jones and Csonka, Am. Soc. Biol. Chem. Proc., 26, XXIX (1932).

¹⁰Hartman and Cheng, J. Chinese Chem. Soc., 4, 152 (1936).

¹¹Ryndin, Colloid J. (U.S.S.R.), 2, 811 (1936).

¹²Osborne and Campbell, J. Am. Chem. Soc., 20, 419 (1898).

soybeans as a globulin which they called glycinin. They also found a second globulin which resembled phaseolin, about 1.5% of albumin and a small amount of proteose.

Recently Ryndin¹¹ studied the physical chemistry of soybean proteins. This work involved the preparation of glycinin according to Osborne's procedure, and includes data on the extraction of protein from oil-free soybean meal by sodium chloride solutions of several concentrations. He shows variation in the amount of nitrogenous matter extracted, but no general conclusions concerning salt peptization of soybean meal may be drawn, as he has studied the effect of only one salt.

Since preliminary experiments indicated that a satisfactory study of the nitrogenous constituents of the soybean meal could not be made by following the definitions in the 1908 classification or the procedures of Osborne, it was deemed more profitable to investigate systematically the effect of various neutral salts on the peptization of oil-free meal.

The separation and purification of soybean protein involves its peptization from the meal and its precipitation from the dispersion. The data in the literature for these processes are too fragmentary to be of much value. Horvath's recent publication¹³ on the chemistry of soybean protein extraction, although interesting, presents no additional experimental work. The most extended work in this field is that of Satow,¹⁴ who investigated the separation of soybean protein and studied its properties.

The procedures, as revealed by the patent literature,¹⁵⁻¹⁸ differ little from those described by Satow. The protein is extracted from oil-free meal by dilute alkali or alkaline salts, followed by precipitation with acid. Since the optimum conditions for this procedure are not recorded, it seemed desirable also to study quantitatively the dispersion of nitrogenous constituents

¹³Horvath, Chemistry & Industry, 56, 735 (1937).

¹⁴Satow, Tech. Repts. Tôhoku Imp. Univ., 2, No. 2 (1921).

¹⁵Johnson, U. S. Patent 1,680,264 (1928).

¹⁶Beaufour, U. S. Patent 1,755,531 (1930).

¹⁷Cone and Brown, U. S. Patent 1,955,375 (1934).

¹⁸Burruss and Ruth, U. S. Patent 2,007,962 (1935).

of oil-free soybean meal over a wide range of hydrogen ion concentrations, using suitable acids and bases as dispersing agents, and thus to determine the limitations of the process.

In the investigation of neutral salt solutions as dispersing agents, it was observed that low concentrations of salts, especially those of calcium and magnesium, had a marked inhibiting action on the dispersion of the protein. Hence the action of dilute neutral salts in combination with acids and bases was also studied.

Experimental

Materials.--The large number of varieties of soybeans,¹⁹ their wide geographical distribution, and the variation in their composition,^{20,21} require that one variety of beans should be selected for experimental purposes. Accordingly, Illini soybeans grown under the supervision of the U. S. Bureau of Plant Industry in 1936 near St. Joseph, Illinois, were chosen for use in the present investigation. The beans were stored in a cool dry room from the time of harvest until the samples were prepared; they were then cracked and flaked on laboratory mill rolls and the oil removed by extraction with petroleum ether (boiling point 30-60° C.) in a modified Soxhlet extractor²² holding 8 kg. of beans.

Other seeds selected for comparative study were Wisconsin Pedigree spring barley, 1936 crop; Brill winter wheat, 1936 crop; and Wisconsin Pedigree rye, 1933 crop, all grown at the University of Illinois farm; Bison flax, 1936 crop, raised at Crawfordsville, Indiana; tepary beans, 1936 crop, grown at Brookings, South Dakota; and Alaska peas. These were ground coarsely in a coffee mill, extracted with petroleum ether in a Soxhlet extractor, and then ground in a pebble mill for nineteen hours to pass a 100-mesh screen. The peas were ground directly in a pebble mill to the same fineness, without oil-extraction. Moisture, nitrogen, oil, ash, and phosphorus contents of the extracted meals are given in

¹⁹C. V. Piper and W. J. Morse, "The Soybean," The McGraw-Hill Book Co., New York, 1923, chap. ix.

²⁰Csonka and Jones, J. Agr. Research, 46, 51 (1933).

²¹Bailey, Capen, and LeClerc, Cereal Chem., 12, 441 (1935).

²²Sando, Ind. Eng. Chem., 16, 1125 (1924).

Table 1. The salt solutions used for protein peptizations were made up on a volume normal basis from c.p. or analytical grades of chemicals.

TABLE 1

PERCENTAGE COMPOSITION OF SEED SAMPLES AFTER OIL EXTRACTION^a

Sample	Percentages				
	Moisture	Nitrogen	Oil	Ash	Phosphorus
Soybeans	9.28	7.80	0.35	5.55	0.97
Flax	10.44	6.98	2.26	4.59	0.74
Tepary	10.31	4.42	0.44	3.18	0.41
Barley	10.47	1.91	0.92	2.58	0.18
Rye	11.70	2.31	0.78	1.72	0.18
Wheat	11.97	2.23	0.57	1.61	0.14
Peas	8.43	3.80	0.95	2.77	..

^a Analytical results are the average of two or more determinations.

Meal Particle Size.--Preliminary experiments showed a marked variation in the amount of nitrogenous matter extracted with a variation in meal particle size. A close examination of the ground meal also shows a wide variation in particle size for any particular grinding procedure, due largely to the toughness of the seed coat. Since there is very little information in the literature on this subject,^{4,23} it was necessary to establish a satisfactory size for the present investigation. Three different samples were obtained from flaked soybean oil-free meal by grinding in a Wiley mill through the 2, 1, and 0.5 mm. screens respectively, and a fourth sample was ground in a quart size pebble mill for nineteen hours. A sieve analysis of each sample is given in Table 2. These samples were extracted with normal solutions of sodium chloride and sodium sulfate, with half normal solutions of the four sodium halides, and with water. The results are given in Table 3 in terms of per cent of total nitrogen extracted for the various size gradations used. All determinations were run in duplicate by the A. O. A. C. Kjeldahl-Gunning-

²³ Bishop, J. Inst. Brewing, 35, 316 (1929).

TABLE 2

SIEVE ANALYSIS OF 100-GRAM SAMPLES OF SOYBEAN MEAL

Wire Mesh Rating	Grams			
	Wiley Mill 2 mm.	Wiley Mill 1 mm.	Wiley Mill 1/2 mm.	Pebble Mill 19 hours
- 20	0.3	..	..	..
20- 40	29.2	4.4	..	..
40- 60	38.0	37.0	..	..
60- 80	10.8	18.4	12.4	..
80-100	19.8	5.0	7.0	4.4
100-200	..	16.6	27.8	3.2
200-	..	17.0	51.0	89.0
Total	98.1	98.4	98.2	96.6

TABLE 3

TOTAL NITROGEN EXTRACTED FROM SOYBEAN MEAL OF DIFFERENT DEGREES OF FINENESS BY WATER AND VARIOUS SALT SOLUTIONS

Type of Grinding	Percentages						
	Water	N NaCl	N Na ₂ SO ₄	0.5N NaF	0.5N NaCl	0.5N NaBr	0.5N NaI
Wiley 2 mm.	83.3	75.4	73.1	..	..	..	..
Wiley 1 mm.	84.3	79.3	76.5	..	..	..	..
Wiley 1/2 mm.	89.4	84.9	81.7	63.1	84.0	86.9	87.0
Pebble mill	91.0	80.5	76.8	62.8	76.9	81.7	84.0

Arnold method with a precision of 1% or better for the percentage total nitrogen extracted. The non-protein nitrogen was not determined, but has been reported by several workers²⁴⁻²⁶ with va-

²⁴A. L. Winton and K. B. Winton, "Structure and Composition of Foods," John Wiley & Sons, New York, Vol. I, 1932, Vol. II, 1935.

²⁵Hamilton, Uyei, Baker, and Grindley, J. Am. Chem. Soc., **45**, 815 (1923).

riation from 2.88 to 10.95% of the total nitrogen.

Extraction Procedure.²⁷--Two and one-half grams of the meal and 100 ml. of the dispersing solution were placed in a 250-ml. centrifuge bottle and shaken mechanically for thirty minutes. The bottles were centrifuged for six minutes in a centrifuge developing a maximum relative centrifugal force at the bottle tip of 1,975 times gravity. In the case of dispersions containing acid or base, with or without added salts, a 10 ml. aliquot of the supernatant liquid was removed for nitrogen analysis. In the case of the neutral salt solutions the entire supernatant liquid was decanted into a 500-ml. volumetric flask, to which was added the supernatant liquids from second and third extractions made likewise, but with a ten-minute shaking period. Longer periods of shaking or centrifuging did not change the results. The combined extracts were diluted to 500 ml. and a 50 ml. aliquot taken for nitrogen analysis. No precipitation occurred on dilution, although in the case of some of the calcium chloride extracts a slight cloudiness appeared. The meal ground through the 2-mm. screen did not pack well on centrifuging; in this case the aliquot was taken for analysis after filtering the diluted extract through coarse filter paper. All other meals packed well. The temperatures of the extracting solutions varied from 24-33° C. The temperature coefficient²⁷ in this range is very small and does not affect the results.

The experiments using the three different size gradations from the Wiley mill indicate that the quantity of nitrogenous constituents extracted from the meal by all dispersing solutions used increases with a decrease in particle size. The results from the meal ground in the pebble mill, which has the smallest average particle size, agree well with the results for the other meal samples in the case of water, but not for the salt solutions used, which show a lower value for the quantity of nitrogen extracted. This difference amounts to 7.1% for the half-normal sodium chloride solution. This contradictory behavior may be accounted for

²⁶Becker, Milner, and Nagel, Cereal Chem., 17, 447 (1940).

²⁷Certain of the extraction technics used in this investigation were adopted from the work of Nagel, Becker, and Milner, on "Some Physical Factors Affecting the Dispersion of Soybean Protein in Water," Cereal Chem., 15, 463 (1938).

by the different grinding action of the two mills; i.e., the Wiley mill has a cutting action, while the pebble mill has a crushing or pounding action.

On the basis of the above results the meal selected for further study was that obtained by grinding in the Wiley mill through the 0.5 mm. screen or by grinding in a pebble mill to pass a 100-mesh sieve. In any one series of determinations the same meal was used throughout.

Effect of Meal Age.--The well-known instability of protein systems² suggested the desirability of determining, after various intervals of time, the percentage of nitrogen and of total water-extractable nitrogen in the ground soybean meal samples.²⁸ The results are given in Table 4 as well as data on a few extractions with 0.5 N sodium chloride solutions. These results show a small increase (from 7.80 to 8.05%) in the nitrogen content of sample A which was ground in June, 1937. This increase may be accounted for by a corresponding loss of moisture. Sample B was flaked and extracted at the same time as sample A but was not ground until October, 1937. Its nitrogen content remained constant at 8.14%.

Although the effect of age on the nitrogen content of the meal is apparently negligible, this is not true for the water-extractable nitrogen which shows a steady decrease with aging. This decrease amounts to approximately 1.1% per month under the conditions of storage and for the time interval covered, as determined by the single extraction technic. The decrease in water-extractable nitrogen by the technic of three successive extractions is much less--namely, about 0.5% per month.

The few available data on extractions by 0.5 N sodium chloride indicate, upon aging of the meal, a larger change (decrease) in salt-extractable nitrogen than in water extractable nitrogen. The previous section showed that the amount of salt-extractable nitrogen from meal ground in a pebble mill was much less than that obtained from meal ground in the Wiley mill, although the water-extractable nitrogen was about the same for the two meals. It appears that intensive grinding and aging have

²⁸ During the course of this investigation Jones and Gersdorff reported on "Changes That Occur in the Protein of Soybean Meal As a Result of Storage," J. Am. Chem. Soc., 60, 723 (1938).

TABLE 4

EFFECT OF AGING OF MEAL ON THE QUANTITY OF EXTRACTABLE NITROGEN

Sample	Date of Extn.	% N in Meal at Date of Extn.	% N Extracted by			
			H ₂ O		NaCl	
			Three 100-ml. Extns.	One 100-ml. Extn.	Three 100-ml. Extns.	One 100-ml. Extn.
A ^a	6- 7-37	7.80	90.1	..	84.1	..
	7-29-37	7.80	..	85.5	..	..
	12- 4-37	7.80	..	81.1	..	..
	2-21-38	8.05	86.2	79.7	71.6	69.1
B ^a	10- 7-37	8.14	90.0	84.8	..	..
	11-19-37	8.14	..	82.6	..	..
	12- 4-37	8.14	..	81.8	..	72.4
	1-10-38	8.14	..	80.7	..	..
C ^b	2-21-38	7.31	89.9	85.1	84.3	80.8

^aSamples A and B taken from the same lot of beans of the 1936 crop.

^bSample C same variety as A and B but from 1937 crop.

somewhat similar effects on the quantity of nitrogen that can be extracted from the meal.

The data in Table 4 indicate that the decrease in extractable nitrogen with aging is greater in the ground meal than in the flaked meal. However, the storage conditions of the two meals were different and may be responsible for this discrepancy. The ground meal was stored in glass bottles exposed to diffuse light, whereas the flaked meal was stored in cans protected from light.

In view of the fact that the nitrogen extractability of the meal decreases with age, the time for the collection of data for any single dispersion curve was limited to one week where possible.

Recently Jones and Gersdorff²⁸ reported that "the amount of nitrogen which can be extracted (from soybean meal) with neutral salt solutions decreases with the aging of the meal." They also reported a decrease in the digestibility of the nitrogen in vitro.

Water was used as the extracting solution for most of the

observations on aging recorded in this paper. Consequently the data obtained are not directly comparable with those of Jones and Gersdorff. However, the few salt solution extractions made agree well with their data, despite differences in technic.

Jones and Gersdorff also showed the effect of temperature on the stability of the protein. Other factors which may markedly influence this stability are the moisture content of the meal and the influence of light.

Protein Peptization by Neutral Salts.--The effect of 12 different neutral salts on the peptization of nitrogenous constituents from soybean meal was determined, using the meal and the procedure outlined previously. The pH values of the salt dispersions were taken on a portable glass electrode pH meter. Results are given in Fig. 1 and Tables 5 and 6.

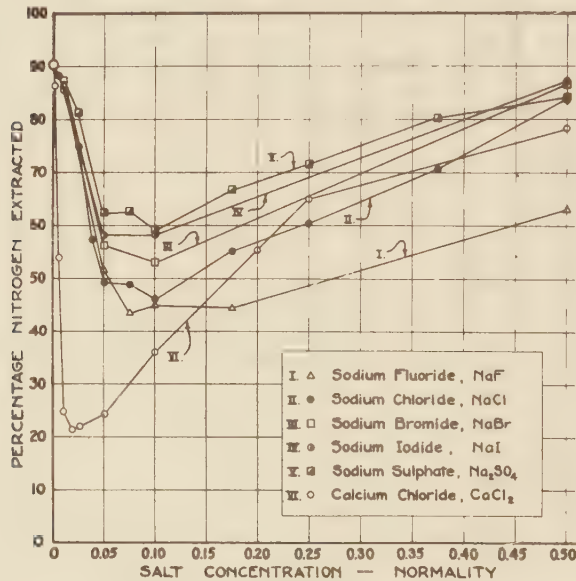


Fig. 1.--Total nitrogen dispersed from solvent-extracted soybean meal by various salts.

The writer knows of no published study on other seeds or grains which has recorded as high a water peptization of nitrogenous constituents as is found with soybean meal. This observation

TABLE 5
TOTAL NITROGEN EXTRACTED FROM SOYBEAN MEAL BY VARIOUS SALT SOLUTIONS^a

Normality of Solution	Percentages											
	LiCl	NaF	NaCl	NaBr	NaI	Na ₂ SO ₄	Na ₂ C ₂ O ₄	Na ₂ C ₄ H ₄ O ₆	KCl	CaCl ₂	MgCl ₂	MgSO ₄
.001	..	..	88.6	..	..	..	..	..	..	86.8	87.1	..
.005	..	..	87.0	87.5	85.7	87.1	88.6	88.7	87.7	54.4	56.7	..
.010	86.7	86.3	..	..	..	..	..	..	..	25.3	31.9	36.8
.0175	..	..	..	..	..	81.3	..	..	..	21.7	..	..
.025	..	..	75.1	..	..	..	..	..	..	22.1	23.6	..
.0375	..	..	57.6	..	..	..	..	..	..	..	..	..
.050	46.2	51.8	49.2	56.4	58.3	62.5	76.4	62.1	56.6	24.7	24.8	27.3
.075	..	43.6	48.6	..	..	62.8	..	..	..	..	..	..
.100	42.1	45.3	46.1	53.2	58.3	59.3	63.6	55.8	52.3	36.1	31.7	36.7
.175	..	44.6	55.0	..	..	66.7	..	..	..	55.6	..	..
.250	..	..	60.7	..	..	71.8	..	..	..	65.1	67.4	..
.375	..	..	70.9	..	..	80.6	..	..	..	..	..	..
.500	82.2	63.1	84.0	86.9	87.0	84.4	79.0	79.6	86.0	73.4	78.2	80.8
1.0	..	..	86.0	..	..	82.0	..	..	..	77.5	..	..
2.0	..	..	82.2	..	..	75.9	..	..	..	75.9	..	..
3.0	..	..	78.9	..	..	67.1	..	..	..	..	..	..
Saturated	..	..	73.6	..	..	..	..	..	..	..	..	..

^aWater extraction gives 90.1% nitrogen.

led the writer to extend the salt peptization experiments to dilute as well as concentrated salt solutions. Examination of the data shows that, for soybean meal, salt solutions do not peptize as much nitrogenous matter as water, and that this inhibiting action is greater in dilute solutions. A minimum dispersion for univalent cations occurs at about 0.1 N, and for divalent cations at about 0.02 N.

To determine whether any other seed exhibits the peculiar type of curve shown by the soybean, peptization studies were made on wheat, rye, and barley grains, flax seed, and tepary beans, using sodium chloride solutions. The procedure and meals used have been described above. The results, as given in Fig. 2 and Table 7, show that the curves for wheat, rye, barley, and flax agree in form with those found in the literature, but that the tepary bean behaves like the soybean, i.e., water peptizes almost 90% of the total nitrogen, water peptization is greater than that for any salt concentration used, and a minimum occurs at about 0.04 N for sodium chloride.

Action of Salts on Water Extract.--Soybean meal was extracted with water in the manner outlined above, but the combined extracts (three successive extractions) were diluted with enough water and calcium chloride to make the 500 ml. of the final solution 0.0175 N with respect to the calcium ion. A white flocculent precipitate formed immediately. The mixture was shaken and filtered, and an aliquot was taken for analysis. While water peptizes 90.1% total nitrogen, this filtered solution contained only 20.7% of the total nitrogen in the amount of meal used. This value is in close agreement with the 21.7% total nitrogen peptized by 0.0175 N calcium chloride in the regular procedure.

In the same manner when sodium chloride is added to a water-peptized soybean meal solution to make it 0.1 N, a similar white flocculent precipitate forms, but at a much slower rate than with calcium chloride. The filtered solution contained 53.1% total nitrogen as compared with 46.1% total nitrogen peptized by 0.1 N sodium chloride in the regular procedure.

Table 8 contains the above data as well as the per cent total nitrogen peptized by three separate successive extractions of a soybean meal sample by 0.1 N sodium chloride, by 0.0175 N calcium chloride, and by water. Also given in the table is the per cent total nitrogen left in solution after flocculation of

TABLE 6
pH OF PROTEIN DISPERSIONS IN SALT SOLUTIONS^a

Normality of Solution	LiCl	NaF	NaCl	NaBr	NaI	Na ₂ SO ₄	Na ₂ C ₂ O ₄	Na ₂ C ₄ H ₄ O ₆	KCl	CaCl ₂	MgCl ₂	MgSO ₄
0.001	..	6.6	..	6.5	..	..	6.8	..	..	6.6	6.6	..
0.010	6.5	6.6	..	6.5	..	6.5	6.8	6.6	6.5	5.7	6.1	6.2
0.050	6.4	6.5	6.4	6.5	6.5	6.5	6.7	6.5	6.4	5.5	5.9	6.0
0.100	6.3	6.5	6.2	6.4	6.5	6.1	6.7	6.5	6.3	5.4	5.8	5.9
0.500	6.0	6.5	6.1	6.5	6.5	6.1	6.8	6.5	6.0	5.2	5.6	5.7
1.000	..	..	6.0	..	..	..	..	..	..	5.2	..	..

^apH of water extract, 6.7.

TABLE 7
TOTAL NITROGEN EXTRACTED FROM OIL-FREE MEAL BY SODIUM CHLORIDE

Meal	Percentages										
	0.000	0.005	0.010	0.025	0.0375	0.050	0.0625	0.075	0.100	0.175	0.500 1.00
Barley	18.9	20.5	..	22.9	..	23.6	..	23.9	24.9	26.9	30.2 31.2
Rye	44.5	49.6	..	53.5	..	56.1	..	58.2	58.5	59.4	47.3 47.3
Wheat	30.3	32.1	..	37.5	..	40.2	..	40.8	41.9	43.8	44.8 45.2
Tepary	90.0	84.0	78.6	72.5	69.7	71.7	76.8	77.5	80.6	86.7	88.6 88.5
Flax	65.6	67.5	..	72.6	..	74.6	..	75.6	77.6	80.5	83.3 84.0

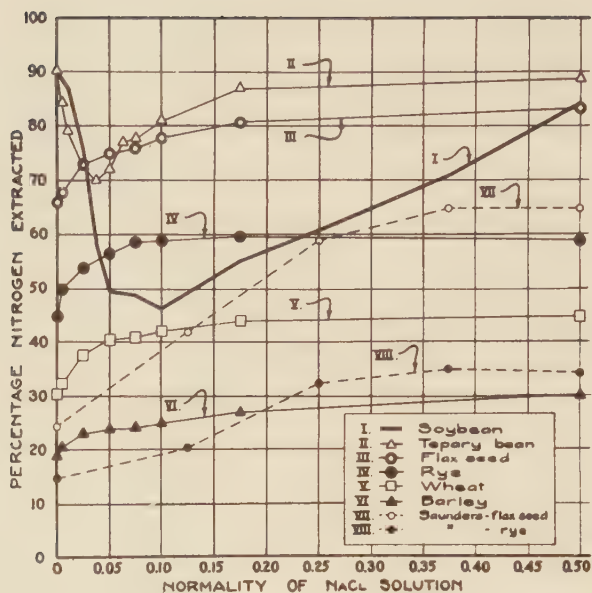


Fig. 2.--Total nitrogen dispersed from various seed meals by sodium chloride solutions.

three separate successive water extracts of a meal sample, by addition of enough calcium chloride to make the water extracts 0.0175 N in respect to this salt, and by addition of enough sodium chloride to make them 0.1 N.

Protein Peptization by Acids and Bases with and without Added Salts.--The first series of extractions was made with hydrochloric, trichloroacetic, sulfuric, oxalic, and phosphoric acids, and with sodium and calcium hydroxides at various concentrations to determine the effect of hydrogen-ion concentration on the amount of total nitrogen dispersed. These data are recorded in Tables 9 and 10 and plotted in Fig. 3.

A second series of extractions was made using 0.01, 0.1, and 0.5 N solutions of sodium chloride in which the pH was varied by addition of hydrochloric acid or sodium hydroxide. These data are shown in Table 11 and Fig. 4.

A third series of extractions was carried out, using 0.001, 0.0175, 0.1, and 0.5 N solutions of calcium chloride in

TABLE 8
PERCENTAGE NITROGEN EXTRACTED BEFORE AND AFTER FLOCCULATION

Number and Volume of Extract	Percentages				
	Water Extract before Flocculation	Addition of CaCl ₂ to Water Extract until .0175 N	Extracted with 0.0175 N CaCl ₂	Addition of NaCl to Water Extract until 0.1 N	Extracted with 0.1 N NaCl
First extract Volume 93 ml.	79.0	16.3	16.0	51.2	38.2
Second extract Volume 100 ml.	8.8	1.8	2.5	5.2	4.4
Third extract Volume 100 ml.	1.6	0.5	1.0	1.4	2.2
Sum of three separate extracts	89.4	18.6	19.5	57.8	44.8
Combined extracts diluted to 500 ml.	90.1 ^a	20.7	21.7 ^a	53.1	46.1 ^a

^aPlaced in table for comparison.

TABLE 9
TOTAL NITROGEN EXTRACTED FROM OIL-FREE SOYBEAN MEAL BY ACIDS

Hydrochloric			Trichloroacetic			Sulfuric			Phosphoric			Oxalic		
Me. ^a Acid/g. Meal	pH	% N Dis- persed	Me. Acid/g. Meal	pH	% N Dis- persed	Me. Acid/g. Meal	pH	% N Dis- persed	Me. Acid/g. Meal	pH	% N Dis- persed	Me. Acid/g. Meal	pH	% N Dis- persed
0.0	6.6	84.8	0.0	6.6	84.8	0.0	6.6	85.5	0.0	6.6	84.8	0.0	6.6	84.8
0.073	6.2	64.7	0.092	6.0	60.8	0.004	6.6	84.3	0.098	6.4	75.0	0.097	6.0	61.5
0.146	5.8	37.1	0.184	5.5	22.2	0.016	6.5	82.8	0.175	6.2	66.0	0.194	5.5	27.6
0.293	5.1	12.7	0.368	4.8	11.0	0.040	6.3	75.7	0.350	5.8	48.6	0.389	4.9	11.0
0.440	4.6	9.3	0.508	4.5	8.5	0.082	6.0	65.9	0.456	5.5	22.9	0.584	4.4	7.4
0.584	4.2	8.5	0.60	4.2	7.9	0.122	5.8	53.6	0.876	5.0	12.8	0.776	4.1	6.7
0.732	3.8	10.5	0.736	3.8	8.1	0.163	5.6	31.7	1.052	4.8	11.6	0.972	3.8	7.4
0.916	3.3	51.0	0.920	3.3	10.2	0.245	5.2	15.8	1.316	4.6	10.1	1.216	3.4	9.5
1.100	2.9	72.6	1.060	3.0	24.4	0.326	4.9	13.4	1.752	4.1	9.1	1.460	3.2	15.8
1.464	2.3	83.3	1.244	2.6	44.1	0.400	4.7	11.8	2.188	3.7	10.7	1.944	2.8	62.9
2.196	1.7	84.2	1.380	2.4	50.9	0.408	4.6	11.8	2.728	3.4	24.0	2.432	2.5	72.8
2.93	1.5	84.5	1.84	1.8	46.0	0.490	4.5	10.7	3.50	2.9	72.1	3.92	2.3	77.9
3.66	1.3	82.7	2.30	1.6	38.7	0.571	4.2	10.1	4.38	2.7	80.0	3.89	2.0	81.1
7.32	1.0	75.5	2.76	1.5	35.0	0.653	4.0	10.1	8.76	2.1	82.7	4.86	1.7	78.0
10.98	0.8	60.9	3.22	1.2	27.0	0.734	3.8	11.0	13.14	1.9	83.4	9.72	1.3	77.7
18.30	0.5	53.7	3.68	1.2	12.6	0.816	3.6	11.5	17.52	1.7	83.6	14.58	1.2	78.7
..	..	..	4.60	1.1	9.8	0.898	3.4	13.1	21.90	1.6	80.6	25.10	1.0	67.1
..	..	..	9.20	0.8	7.0	1.224	2.7	37.2	..	..	..	..	..	..
..	..	..	13.80	0.7	5.7	1.632	2.3	62.4	..	..	..	..	..	..
..	..	..	18.40	0.5	5.3	2.448	1.9	68.4	..	..	..	..	..	..
..	..	..	40.0b	..	3.8	2.856	1.6	69.3	..	..	..	..	..	..
..	..	..	68.0b	..	4.9	4.00	1.5	70.3	..	..	..	..	..	..
..	..	..	160.0b	..	93.9	..	..	..	..	..	..	..	..	..

^aMilliequivalents.^bData from paper by Becker, Milner, and Nagel.²⁶

TABLE 10

TOTAL NITROGEN EXTRACTED FROM OIL-FREE MEAL
BY SODIUM AND CALCIUM HYDROXIDES

NaOH			Ca(OH) ₂		
Me. ^a Base/ g. Meal	pH	% N Dispersed	Me. Base/ g. Meal	pH	% N Dispersed
0.017	6.8	84.6	0.0	6.6	82.6
0.084	7.3	87.7	0.007	6.6	81.9
0.168	8.3	91.5	0.014	6.6	82.5
0.420	10.5	93.9	0.028	6.7	83.7
0.840	11.4	95.8	0.042	6.8	83.8
1.68	12.2	96.5	0.056	6.9	83.7
2.94	..	95.2	0.070	6.9	84.7
4.20	..	95.5	0.141	7.3	86.0
..	..	..	0.176	7.7	87.0
..	..	..	0.352	9.8	89.2
..	..	..	0.704	10.8	92.2
..	..	..	1.056	11.2	91.5
..	..	..	1.76	11.7	86.9

^aMilliequivalents.

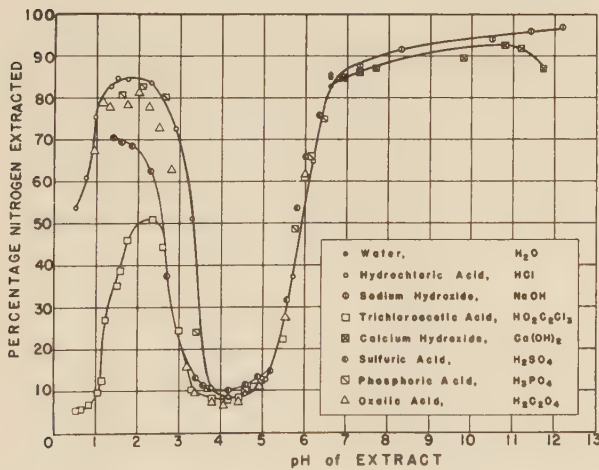


Fig. 3.--Total nitrogen extracted
from oil-free soybean meal by acids and bases.

TABLE 11

TOTAL NITROGEN EXTRACTED WITH SODIUM CHLORIDE SOLUTIONS
IN WHICH THE pH IS VARIED BY THE ADDITION OF
HYDROCHLORIC ACID OR SODIUM HYDROXIDE

Me. ^a of Acid or Base/g. of Meal	0.01 N NaCl		0.1 N NaCl		0.5 N NaCl	
	pH	% N Extd.	pH	% N Extd.	pH	% N Extd.
		Hydrochloric Acid Added				
0	6.5	68.9	6.4	39.9	6.1	73.8
0.080	..	..	6.0	30.4	5.6	70.4
0.160	5.5	22.4	5.5	23.7	5.3	70.3
0.320	4.9	13.5	5.0	17.3	4.8	57.9
0.560	4.2	10.7	4.2	14.4	4.1	42.3
0.800	3.5	13.3	3.4	27.2	3.6	60.5
1.000	3.1	65.3	3.2	51.6	3.1	67.7
1.200	2.8	78.3	2.7	71.4	2.7	69.6
1.600	..	..	2.1	76.3	2.0	68.6
2.00	1.8	82.1	..	..	..	..
2.40	..	..	1.5	76.3	1.5	67.7
4.00	1.2	77.7	1.2	74.9	1.1	67.2
8.00	0.9	72.6	0.8	70.4	0.7	64.9
12.00	0.5	65.0	0.7	56.7	0.5	63.6
16.00	0.3	52.1	0.5	53.9	0.4	61.5
		Sodium Hydroxide Added				
0.016	..	..	..	..	6.2	72.4
0.032	6.8	79.2	6.7	43.9	6.4	73.3
0.112	..	..	7.3	51.7	7.0	75.2
0.241	8.9	90.3	9.1	67.8	8.7	80.5
0.802	11.1	94.9	11.3	91.7	11.2	91.4
2.00	..	95.0	..	93.3	..	93.3
3.21	..	95.2	..	94.8	..	93.3

^aMilliequivalents.

which the pH was varied by the addition of hydrochloric acid or calcium hydroxide. Results of this series are given in Table 12 and Fig. 5. A fourth series of extractions was made in order to compare the dispersion of soybean protein with that of other vegetable proteins by the same technic. In this series wheat, tepary beans, and Alaska peas were extracted with hydrochloric acid and sodium hydroxide solutions of various concentrations. These data are given in Table 13. All pH measurements were made on the glass electrode.

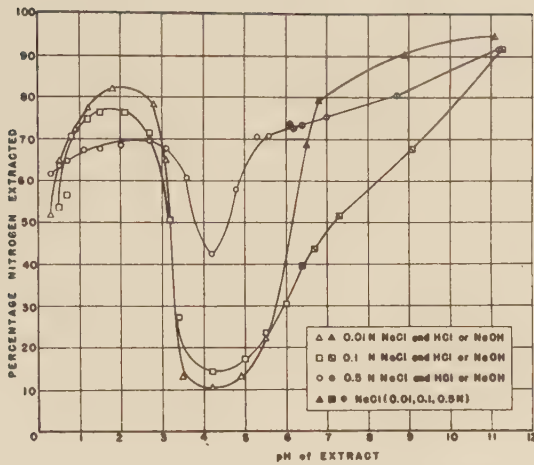


Fig. 4.--Total nitrogen extracted by sodium chloride solutions in which the pH is varied by addition of hydrochloric acid or sodium hydroxide.

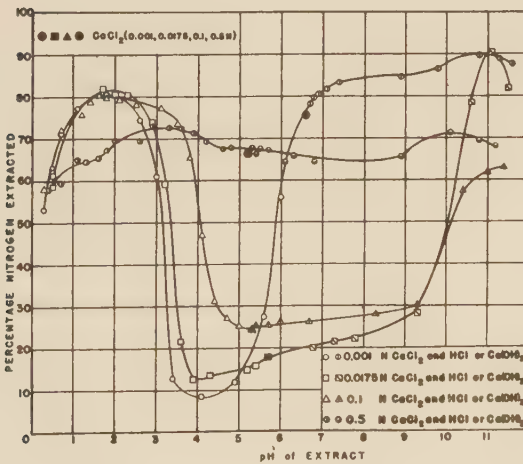


Fig. 5.--Total nitrogen extracted by calcium chloride solutions in which the pH is varied by addition of hydrochloric acid or calcium hydroxide.

TABLE 12

TOTAL NITROGEN EXTRACTED WITH CALCIUM CHLORIDE SOLUTIONS
IN WHICH THE pH IS VARIED BY THE ADDITION OF
HYDROCHLORIC ACID OR CALCIUM HYDROXIDE

Me. ^a of Acid or Base/g. of Meal	0.001 N CaCl ₂		0.0175 N CaCl ₂		0.100 N CaCl ₂		0.500 N CaCl ₂	
	pH	% N Extd.	pH	% N Extd.	pH	% N Extd.	pH	% N Extd.
	Hydrochloric Acid Added							
0	6.6	75.5	5.7	17.9	5.3	24.9	5.2	66.3
0.0800	6.0	56.0	5.4	15.8	5.0	25.3	4.8	67.9
0.1598	5.6	27.4	5.2	14.9	4.7	27.1	4.6	67.5
0.3197	4.9	11.9	..	..	4.4	31.2	4.2	69.2
0.4795	..	..	4.3	13.3	4.1	47.1	3.9	71.1
0.5594	4.1	8.5	..	..	..	..	..	..
0.6394	..	..	3.9	12.7	3.8	65.6	3.6	72.5
0.7992	3.4	12.8	3.6	21.3	3.5	73.8	3.3	72.7
0.9920	3.0	61.0	3.2	59.1	3.1	77.5	2.9	73.0
1.199	2.6	74.4	2.9	74.0	2.5	78.1	2.6	69.6
1.598	..	..	2.3	80.5	2.1	79.6	2.0	69.7
1.998	1.8	80.7	2.0	80.8	1.8	79.8	1.8	67.3
2.398	..	..	1.7	82.0	1.6	80.4	1.6	65.5
3.197	..	..	1.4	..	1.4	79.0	1.3	64.6
3.996	1.1	77.2	1.2	..	1.2	76.6	1.1	65.1
7.990	0.7	71.3	0.8	..	0.7	72.3	0.7	59.6
11.99	0.5	63.1	0.5	61.4	..	..	..	..
15.98	0.3	53.1	0.5	58.8	0.3	58.1	0.4	58.0
	Calcium Hydroxide Added							
0.014	..	..	5.7	17.6	..	..	..	..
0.018	6.6	76.2	..	..	5.4	25.5	5.3	67.8
0.035	6.7	78.3	..	..	5.5	25.5	5.4	66.3
0.053	6.8	79.8	..	..	5.6	25.7	5.5	67.5
0.070	6.9	80.7	..	..	5.7	25.7	5.7	67.1
0.106	7.1	81.9	..	..	6.0	26.4	6.1	64.3
0.141	7.4	83.3	6.8	20.0	6.7	26.3	6.3	65.9
0.176	..	..	7.3	21.3	..	..	6.8	64.4
0.211	..	..	7.8	22.1	..	..	..	..
0.246	8.9	84.7	..	..	8.3	28.1	..	..
0.352	9.8	86.5	9.3	28.2	9.3	30.3	8.9	65.6
0.704	10.8	89.9	10.6	78.5	10.4	57.8	10.1	71.1
1.06	11.3	89.0	11.1	90.4	11.0	62.0	10.8	69.4
1.41	11.6	87.7	11.5	81.9	11.4	63.2	11.2	68.0

^a Milliequivalents.

TABLE 13

TOTAL NITROGEN EXTRACTED FROM ALASKA PEA, TEPARY
BEAN, AND WHEAT MEALS BY HYDROCHLORIC
ACID AND SODIUM HYDROXIDE

Me. ^a Acid or Base/g. Meal	Alaska Peas		Tepary Beans		Wheat	
	pH	% N Extd.	pH	% N Extd.	pH	% N Extd.
Hydrochloric Acid Extraction						
1.181	2.0	82.8	2.0	86.3	1.7	50.9
0.551	2.8	51.9	3.1	69.9	2.1	47.1
0.315	3.9	14.8	4.0	22.7	2.5	42.8
0.1576	4.9	19.3	4.9	24.7	3.5	23.1
0.0000	6.7	77.9	6.5	78.2	6.4	20.2
Sodium Hydroxide Extraction						
0.0802	8.0	94.7	7.6	93.2	9.1	54.2
0.1603	9.1	97.6	8.7	94.9	10.1	89.0
0.401	10.5	97.7	10.4	99.3	11.0	97.3

^aMilliequivalents.

Discussion

Effect of Particle Size.--In Fig. 2 are included dispersion curves for flaxseed and rye taken from the data of O'Hara and Saunders.⁵ These curves are consistently much lower than the corresponding curves plotted from the results of this study, e.g., for water extraction of flax seed Saunders found 24.27% of the total nitrogen, whereas 65.6% total nitrogen was obtained in the present investigation. Gortner,⁴ on the other hand, found 64.07% total nitrogen for water extraction of flax seed. Considering differences in variety and technic, the results reported herein agree well with those of Gortner. It was noted that Gortner ground the entire seed to pass a hundred mesh screen and used the resulting whole meal. This procedure is very similar to the one used in this study. An examination of Saunders' papers disclosed the fact that after the seed was ground in a Wiley mill, and the meal was sifted, a particular sieve fraction was selected for extraction experiments. For flax seed the 80-100 mesh portion was chosen,⁵ and for orange seed meal either the 60-80,⁵ 40-60,⁶ or

20-40⁷ sieve fraction. The portion selected for rye is not mentioned.

On examination of the various fractions of sifted oil-free soybean meal, the major part of the hulls is found in the coarser fractions. For the 1-mm. meal, which contains 7.80% nitrogen, the coarsest fraction, 20-40 mesh, has 6.59% nitrogen, whereas the finest, passing through the 200 mesh screen, has 8.75%. It is also true that the nitrogenous matter of the hull is much less easily extracted by water²⁷ and neutral salt solutions than is that of the remaining seed parts. These facts and the results of the particle size experiments may largely account for the disagreement noted above.

From the biochemical and industrial viewpoints, a study of the chemical and physical differences between the various seed parts of the soybean, such as hull, endosperm and embryo, would be valuable. It is believed, however, that mechanical separation of these seed parts is not accomplished well enough by ordinary grinding and screening processes to be of material value. In the present study it was deemed advisable to use whole meal rather than any particular sieve fraction thereof.

The results on the effect of meal particle size show that the quantity of nitrogenous constituents extracted from soybean meal increases with an increase in number of successive extractions and with a decrease in particle size. There is also an indication that the method of grinding affects the results. The importance of further studies in this phase of protein investigation and the necessity of standardizing procedures and technics is evident.

Effect of Neutral Salts.--The pH of the water extract of soybean meal is 6.7; for the salt solution extracts the values are somewhat lower, the lowest being 5.2 for the normal calcium chloride solution. Since the salt solutions are made up of neutral salts, the pH change that follows the addition of meal to the solution must be the result of some reaction between the meal and the salt solution. An examination of Table 6 shows that the pH decreases with an increase in salt concentration and that this change is largest for the calcium and magnesium chloride solutions. However, the pH change does not correlate with a change in the nitrogen extracted. A more exact valuation of the relative influence of the salt ions and the hydrogen ion is in the

following sections.

The peptization by water and salt solutions of the nitrogenous constituents from soybean and tepary bean meals is unusual in three ways. The amount of nitrogen extracted is greater for water than for any of the salts studied; a very high percentage of their nitrogenous constituents is extracted by water (about 90%); and in dilute solutions there is a sharp minimum in the peptization curve varying with the kind of salt used.

This minimum on the curves for soybean spreads over a wider range of concentrations for the univalent cations than for the divalent cations. For the sodium salts that have been studied in greatest detail: namely, sodium chloride, sodium fluoride, and sodium sulfate, there is indication of a leveling off or even a slight rise between 0.05 and 0.1 N (Fig. 1). This does not appear to be true for the salts of divalent cations. This is correlated with the fact that divalent cations flocculate the protein in an aqueous extract more completely, at a much faster rate, and at lower concentrations than do univalent cations, in accordance with the Hardy-Schulze rule.²⁹ It may help to explain why the flocculation data do not agree with the dispersion data for univalent cations, but do for divalent cations. A more precise technique will be necessary for proper investigation of this concentration range.

The anion action appears to oppose the flocculating action of the cation as is evidenced by a series of sodium salts at 0.05, 0.1 N, and 0.5 N in which the salts of divalent anions disperse more protein than the salts of univalent anions. The order of arrangement of these series changes as the solutions become more concentrated. Thus the valence relationship of both types of anion is most pronounced in the region of the minimum on the curves. This, as well as a lyotropic series of the cations studied, is shown in Table 14.

The curve in the present study for sodium chloride extraction of soybean meal agrees well with that of Ryndin,¹¹ except that he did not investigate the concentration range between 0.0 and 0.4 N. Consequently his curve does not show as low a minimum. His report does not contain a satisfactory description of the

²⁹H. S. Taylor, "Treatise on Physical Chemistry," D. van Nostrand Co., New York, 1931, Vol. 2, p. 1696.

TABLE 14

LYOTROPIC SERIES FOR SOYBEAN PROTEIN IN
ORDER OF DISPERSING ACTION

Normality	Anion Arrangement Sodium Salts	Cation Arrangement Chloride Salts
0.05	Cl < F < Br < I < Tart < SO ₄ < C ₂ O ₄	Mg = Ca < Li < Na < K
0.10	F < Cl < Br < Tart < I < SO ₄ < C ₂ O ₄	Mg < Ca < Li < Na < K
0.50	F < C ₂ O ₄ < Tart < Cl < SO ₄ < Br < I	Ca < Mg < Li < Na < K

technic used, so that a close agreement may be fortuitous. He also gives a sodium chloride dispersion curve for dialyzed soybean meal which is of the same type as the flax and cereal curves shown here. From these two curves he concludes that the high percentage of nitrogenous constituents dispersed from undialyzed soybean meal by water is due to the presence of dialyzable salts in the seed. However, this conclusion is not convincing for the reason that he gives no consideration to the pH changes occurring during dialysis. It is known from work reported in a later section of this dissertation that electrodialysis will lower the pH of a water dispersed soybean meal extract as much as three pH units long before the salts are removed. It may be worth-while to point out also that the theory, that salts normally present in the meal are largely responsible for the protein dispersion, does not agree with the dispersion curves for soybean and tepary bean meals, since added salts up to a limited concentration (minimum on the curves) actually inhibit the dispersion of the protein.

It is not intended to exclude from consideration the action of salts present in the seed as a possible peptizing agent, but rather to include the possibility of additional substances that may act as peptizers. However, a comparison of the proximate and ultimate compositions of soybean and tepary bean with flax seed, rye, wheat, and barley reveals no sufficient differences to account for the unusual behavior of the first two.²⁴ This applies to the ash composition as well as other components.

The hypothesis is suggested that in addition to salts the soybean and tepary bean contain a dialyzable natural peptizing agent not present in the other seeds studied. This peptizing

agent functions upon the addition of water; its dispersing action is adversely affected by the cations of added salts; and after a salt concentration sufficient to neutralize its effect is attained, further salt addition results in the usual salt dispersing action.

From the physical point of view the forces responsible for peptizing and maintaining a protein in its dispersed state are attributed to the charge and hydration of the micelle. Most vegetable proteins in a pure water dispersion extracted from the meal are on the alkaline side of their isoelectric points, i.e., the protein micelles are negatively charged. Since this is true for the wheat, rye, barley, and flax seed studied, which do not exhibit any flocculation in dilute salt solutions, as well as for the soybean and tepary bean, it appears probable that the natural dispersing agent in the last two may be influencing the degree of hydration of their protein micelles. A more thorough knowledge of the various chemical compounds in the soybean, and their properties, may be necessary before a true explanation of its anomalous water and salt dispersion behavior is forthcoming.

Effect of Hydrogen-Ion Concentration.--The pH dispersion curves (Fig. 3) for hydrochloric, sulfuric, oxalic, and phosphoric acids are very similar, showing a minimum dispersion of about 8% of the total nitrogen at a pH of 4.1 to 4.2, and a maximum dispersion of about 83% at a pH of 1.8. As the pH drops below this value, there is a rapid decrease in the amount of nitrogen dispersed. Trichloroacetic acid is considered a protein precipitant^{25,26} and has been used in the determination of nonprotein nitrogen in the soybean. In dilute solution, however, it behaves much like the other acids. The dispersion curve for this acid shows approximately the same minimum point at pH 4.2 as the other curves, but after reaching a maximum of 51% at a pH of 2.3, it drops off rapidly to 5.5% at pH 0.5. Therefore trichloroacetic acid should be used in sufficient concentration to produce a pH below 0.5 if it is employed as a precipitant for soybean protein in the determination of nonprotein nitrogen. Table 9 shows that below this pH the dispersion curve for trichloroacetic acid levels off at approximately 4-5% total nitrogen extracted, and only in very concentrated acid does it rise again.

Becker, Milner and Nagel²⁶ have described "A Method for the Determination of Nonprotein Nitrogen in Soybean Meal," in which they state that there is no clear line of demarcation be-

tween proteins and the nitrogenous nonproteins. However, by comparing the quantity of nitrogenous constituents dialyzable through cellophane membranes in an electrodialysis apparatus with that left in solution after treatment with trichloroacetic acid of various concentrations, they find that the nonprotein nitrogen may be considered as that left in solution by trichloroacetic acid of concentrations between 0.65 and 1.0 N, where it is a minimum.

The most efficient dispersion appears to be produced by sodium hydroxide. The dispersion curve for this base is slightly above that for calcium hydroxide which at higher concentrations points downward quite sharply; this behavior indicates that larger quantities of lime might markedly decrease the amount of nitrogen dispersed.

The acid and base form one continuous curve on which the amount of nitrogenous matter dispersed by water is a single point. The maximum amount of nitrogen dispersed by acid is slightly less than that produced by water, which disperses about 84% by the single extraction technic.

The pH at the minimum point of solubility (approximately pH 4.2) is considered to be an isoelectric point for the protein in soybean meal under the conditions of these experiments. This subject is discussed in detail in a later section.

The data on nitrogen extraction for the Alaska pea and tepary bean in Table 13 are similar to those for the soybean meal; those for the wheat show more resistance to the dispersing action of the acid and alkali.

Combined Effect of Hydrogen-Ion and Salts.--The influence of hydrogen-ion concentration on the salt dispersion of proteins has been given considerable attention in the literature. Loeb³⁰ claimed that at any given pH value the reaction between salts and proteins was stoichiometric and was governed only by the valence of the ion with charge opposite to that of the protein. He believed that the earlier data claiming a lyotropic series was in error because of lack of control of the hydrogen-ion concentration. Gortner³ and others have taken a different point of view, and have produced considerable evidence to support their contention of a lyotropic series effect, especially in relation to the

³⁰J. Loeb, "Proteins and the Theory of Colloidal Behavior," The McGraw-Hill Book Co., New York, 1922.

dispersion of proteins by salts. In extracting protein from wheat flour with 0.5 N magnesium sulfate, Rich⁸ showed that the effect of adding small amounts of acid to the salt was practically negligible.

The present data (Figs. 4 and 5) are intended as a step toward the interpretation of the relative effects of the hydrogen-ion and salt ions on protein dispersion. A previous section indicated that some reaction occurs between the meal and the salt even though no acid is added, as shown by a decrease in the pH value of the extract with an increase in salt concentration (Table 6). This change in pH may be caused by a reaction between the negative protein particles and the cation of the salt, resulting in an increase in the hydrogen-ion concentration. The fact that divalent cations had more effect than monovalent cations would seem to lend support to this hypothesis.

With respect to the superimposed effect of hydrogen-ion on the salt dispersion of the protein, it is clearly evident from these data that at low salt concentrations the effect of hydrogen-ion concentration is greater; at higher salt concentrations (0.5 N for calcium chloride) the effect of pH practically disappears. A concentration of 0.5 N for sodium chloride does not eliminate the hydrogen-ion effect as completely as does 0.5 N calcium chloride, but this effect is very small between pH 5.2 and 8.0. The most pronounced effect of hydrogen-ion is between 3.0 and 5.2, and above 8.0. The trend of the data indicates that at higher sodium chloride concentrations, however, the effect of pH would practically disappear.

The charge on the protein micelle is intimately related to its stability in solution, as shown by loss in dispersibility at the isoelectric point determined by acid precipitation. The minimum point broadens out as the salt concentration is increased and practically disappears for 0.5 N calcium chloride. The question arises as to whether a change in pH is any longer effective in neutralizing the micellar charge in the more concentrated salt solutions, and whether in such solutions the micellar hydration is increased. The answer, at least in part, possibly lies in studies of these systems by the method of electrophoresis (see Part III of this dissertation).

Summary

The amount of protein extracted from soybean meal increases with a decrease in particle size and with an increase in the number of extractions.

Aging of the meal has been found to decrease the quantity of water-extractable nitrogen. This decrease per month for the first few months amounts to about 1.1% of the total nitrogen for a single extraction technic.

A study of the dispersion of the nitrogenous constituents from soybean meal by 12 different neutral salts shows that the neutral salts disperse less of the nitrogenous constituents from the meal than is dispersed by water. This is true also for tepary bean meal, but not for flax seed, rye, wheat, or barley. A minimum occurs in the salt dispersion curves for soybean meal at about 0.1 N for univalent cations, and at about 0.02 N for divalent cations. The sodium chloride dispersion curve for the tepary bean has a minimum at 0.0375 N.

The amount of nitrogenous matter extracted from oil-free soybean meal by hydrochloric, trichloroacetic, oxalic, sulfuric, and phosphoric acids, and by sodium and calcium hydroxides was determined over a wide range of pH values. These data show that the maximum amount of protein extractable by the acids studied is no greater than the amount extractable by water (approximately 84% at 26° C. by the single extraction technic), and that alkali is the most effective dispersing agent, extracting up to 95% of the nitrogenous matter at even moderate concentrations. The acids have a common point of minimum dispersion at pH 4.2, approximately.

Extractions made with salt solutions in which the pH was varied by addition of hydrochloric acid in conjunction with sodium chloride or calcium chloride, show that at low salt concentrations the hydrogen-ion concentration has a greater influence on the amount of protein dispersed than the salt. As the salt concentration is increased, the effect of the acid decreases and practically disappears at 0.5 N for calcium chloride.

PART II

PRECIPITATION OF SOYBEAN PROTEIN

Introduction

The recent rapid increase in the production of soybeans in this country³¹ has made available to industry a cheap domestic protein in practically unlimited quantity. The present production of protein is used mostly in the preparation of cold water paints and of sizes for paper and textiles. However, there is indicated a much broader field of application in the form of adhesives, leather finishes, paper coatings, and plastics.

The domestic supply of soybeans harvested for seed rose from less than 5 million bushels in 1925 to the record high of 91,272,000 bushels in 1939.³¹ The North Central States are the chief producers at present, Illinois accounting for more than half of the yield with 46,820,000 bushels in 1939.³¹ Illinois leads in both acreage planted and average yield per acre. Other important producing states are North Carolina and Mississippi. Recently there has been a progressive increase in the proportion of soybeans processed for industrial purposes, largely because of an active demand for the oil.

There are two procedures for removing the oil--namely, mechanical expression and solvent extraction. Mechanical expression fails to remove about 25% of the oil, and the resulting meal, most of which is used for stock feed, is for various reasons not suitable for protein extraction. The meal from the solvent-extraction process contains approximately 45% protein and less than 1% of oil and is quite suitable for protein extraction.

The present and following studies deal with the various methods of separating the protein from the oil-free meal. Among the first workers in this field, Osborne and Campbell¹² were pri-

³¹Illinois Co-operative Crop Reporting Service, Illinois Department of Agriculture, Division of Agricultural Statistics, Illinois Annual Soybean Report, December, 1940. See also Grove, U. S. Dept. Agr., Tech. Bull. 619 (June, 1938).

marily interested in the identification of the various proteins occurring in the soybean. The glycinin was separated from the meal by extraction with salt, precipitated by dialysis, and dried with alcohol and ether. By this method a white protein product was isolated but in rather low yields. Later Hartman and Cheng¹⁰ modified the Osborne procedure of salt extraction and studied the physical chemistry of glycinin. They also used another procedure in which they extracted with water and salt successively and precipitated the protein by addition of sulfuric or acetic acid.³² Since it was shown in Part I that water is a better extracting agent than salt solutions for removing the protein from the meal, there can be little commercial interest in the Osborne method.

Much of the technological work has been carried out by the Japanese and Chinese workers. Of this group, Satow^{14,33} has been the most prolific writer. His work is too voluminous to be reviewed in detail here; in general, he studied various procedures for the extraction of protein from the meal, precipitation of the protein, and the effect of various chemical treatments on its physical properties, especially those properties which adapt it for the production of plastics. Satow concluded that an alkaline salt with reducing properties, such as sodium sulfite, was best for extracting the protein, although sodium hydroxide gave a higher yield. This alkaline extraction was coupled with acid precipitation. While his reports contain many useful suggestions concerning the refinement of soybean protein and its industrial application, they are principally qualitative in character and suffer from disregard of pH control.

Iinuma and Mashino³⁴ and Mashino³⁵ investigated the influence of pretreatment of the meal on the dispersibility of the protein, especially the effect of heat and different solvents used for the oil extraction. Their work illustrates very well the various factors that might be expected to affect the dispersion of soybean protein, but it is too limited in analytical detail to

³²Cheng and Hartman, J. Chinese Chem. Soc., 4, 149 (1936).

³³Satow, Tech. Repts. Tôhoku Imp. Univ., 3, No. 4 (1923).

³⁴Iinuma and Mashino, J. Soc. Chem. Ind., Japan, 36, Suppl. Binding, 310, 373, 455, 506 (1933).

³⁵Mashino, J. Soc. Chem. Ind., 54, 236 T (1935).

serve as a guide in setting up an industrial process for the production of protein.

Hsieh, Jen, and Chang³⁶ studied protein extraction from soybean oil cake and found sodium carbonate to be a better extracting agent than potassium hydroxide; Chang and Chang,³⁷ working on oil-free soybean meal, determined that potassium hydroxide and sodium hydroxide are of equal value, and that both are superior to sodium carbonate.

In his studies on nitrogen distribution in soybean protein, Friedemann³⁸ extracted soybean meal with 0.2% sodium hydroxide and precipitated the protein by adjusting the solution to 0.1% acidity with acetic acid; he obtained a yield of 82.5% of the total protein. The nitrogen remaining in the solution was partly protein and partly nonprotein, as differentiated by comparing the total nitrogen values found by the Van Slyke and Kjeldahl methods. This work also disregards the effect of pH in the precipitation of protein. There are other articles in the literature pertaining to the separation of protein from the soybean, but their lack of continuity and detail makes them of doubtful value.

Some preliminary work on the electrodialytic purification of soybean protein in an aqueous extract of the meal which had an initial pH of 6.6 revealed a marked drop in pH during the process, accompanied by precipitation of the protein. On continued electrodialysis the pH passed through the isoelectric point to the acid side. Most of the literature on the electrodialysis of proteins has been concerned primarily with the removal of the salts and has paid little attention to the pH changes occurring. The experiments reported here indicate that the isoelectric point of the soybean protein is reached long before appreciable removal of the salts has taken place, and that the precipitation of the protein is caused by the acid formed during the process of electrodialysis. Although some authors³⁹⁻⁴⁴ have noted the appearance

³⁶Hsieh, Jen, and Chang, Chiao-Tung Univ. Research Inst., Ann. Rept. Bur. Chem., 3, 63 (1935).

³⁷Chang and Chang, J. Chem. Eng. China, 4, 177 (1937).

³⁸Friedemann, J. Biol. Chem., 51, 17 (1922).

³⁹Bradfield and Bradfield, J. Phys. Chem., 33, 1724 (1929).

⁴⁰Ettisch and de Loureiro, Biochem. Z., 266, 422 (1933).

of acid on electrodialysis of proteins, only Markovich,⁴² in a study of blood serum, attributed the precipitation of the protein to the acid. Because of this apparent similarity between electro-dialytic and acidic precipitation of soybean protein, a comparative study of the two procedures is included in this report.

Experimental

Materials.--The oil-free meal used in this investigation was prepared from Illini soybeans raised on the University of Illinois farm in 1937. These were treated in the same manner as outlined for the 1936 beans in Part I, ground in a pebble mill to pass a 100-mesh screen and stored in glass bottles in a refrigerator. Although no reliable quantitative data are available on the heat denaturation of soybean protein, it is known that the exposure of the meal to elevated temperatures will alter its dispersibility in water and salt solutions.^{34,35} Therefore room temperature (25-30° C.) was chosen as a standard for the present work. Somewhat different results would be expected for a heated or toasted meal. A proximate analysis of the meal follows: moisture 10.50%, nitrogen 7.14%, and ash 6.03%. The chemicals used were c.p. or analytical grade.

Precipitation by Acids.--In Part I water, alkaline salts, alkalis, and acids were shown to be suitable for the dispersion of soybean protein from the oil-free meal. In the present study each of these was used as a dispersing agent. The protein was precipitated from the alkaline or aqueous dispersions by the addition of various acids, and from the acid dispersion by sodium hydroxide. The precipitates were removed by centrifuging. The difference between the percentage of nitrogen originally present in the dispersion and that left after the removal of the precipitate gave a measure of the amount of protein in the precipitate.

In making up the dispersions, 25 g. of meal were extracted successively with 500 and 300 ml. of the dispersing agent; after

⁴¹Kratz, Kolloid Z., 80, 33 (1937).

⁴²Markovich, J. Applied Chem. (U. S. S. R.), 8, 1444 (1935).

⁴³Murray, J. Soc. Chem. Ind., 55, 302 T (1936).

⁴⁴Watson, Ind. Eng. Chem., 26, 640 (1934).

each extraction the dispersion was centrifuged with a relative centrifugal force of 1975 times gravity at the bottle tip. The two extracts were combined and recentrifuged to remove small particles carried over on decantation, and diluted to 1000 ml. The protein was precipitated by adding varying amounts of acid to 50-ml. aliquots of the dispersion placed in 100-ml. volumetric flasks. A few drops of isoamyl alcohol were added to prevent foaming, and the volumes were brought to 100 ml. by dilution with water. After vigorous shaking, the precipitates formed were removed by centrifuging in 50-ml. tubes with a relative centrifugal force of 2120 times gravity at the tip; 20 ml. of the centrifugate were taken for analysis by the official A. O. A. C. Kjeldahl-Gunning-Arnold method, and the pH of each solution was determined with a portable glass electrode pH meter. This procedure was employed for the following combinations of dispersing and precipitating agents: Water-sulfuric acid, water-hydrochloric acid, water-phosphoric acid, water-trichloroacetic acid, 0.05 N $\text{Na}_3\text{PO}_4\text{-H}_3\text{PO}_4$, 0.05 N Na_3PO_4 -sulfuric acid, 0.05 N sodium sulfite-sulfuric acid, and 0.05 N sodium hydroxide-sulfuric acid. Table 15 gives the results of experiments in which water was the dispersing agent, and Table 16 gives data for the others. Fig. 6 shows the curves.

Precipitation by Electrodialysis.--In the electrodialysis experiments the dispersions were prepared by extracting 50 g. of the meal with 1600 ml. of water, as in the previous section. After dilution to 2000 ml., the extract was placed in a three-chamber, distilled-water electrodialyzer equipped with parchment paper membranes and platinum electrodes and cooled with running water. The potential of the direct current applied was initially 110 volts and gave a current of 0.5 ampere but was raised to 220 volts when the current had dropped to 0.1 ampere. The pH of the solution was determined from time to time with a glass electrode. The temperature of the extract, usually below that of the room, was not very favorable for the growth of bacteria, which was further discouraged by the addition of a few drops of toluene. The appearance of a precipitate was observed when the pH had dropped to approximately 5.2. This value was reached within 2 to 3 hours after the current was started. At the end of a predetermined time the current was shut off and the precipitate removed by centrifuging. An aliquot of the centrifugate was taken for analysis and the amount of protein precipitated was determined by the difference

between the initial and final nitrogen percentage values. This general procedure was repeated several times, progressively lowering with each run the pH value at which the electrodialysis was discontinued. It required about 8 hours by this technic to reach the lowest pH value of 3.1, which was maintained without change on electrodialyzing for 31 hours longer. Table 15 and Fig. 6 give data for a number of these runs.

TABLE 15
PRECIPITATION OF SOYBEAN PROTEIN FROM WATER DISPERSIONS

pH of Soln.	% N ^a Left in Dispersion after Precipitation by:				
	Electro-dialysis	HCl	H ₂ SO ₄	H ₃ PO ₄	Cl ₃ C ₂ O ₂ H
6.6 ^b	86.2 ^b	86.2	86.2	86.2	86.2
6.2	82.4	..	..	..	..
6.0	..	79.4	82.3	..	..
5.6	..	..	..	34.1	43.0
5.5	..	23.5	28.8	..	..
5.4	18.6	..	..	20.5	..
5.2	12.2	..	..	..	19.8
5.1	12.1	16.5	17.4	..	..
4.8	..	14.5	..	14.8	17.6
4.7	10.2	..	..	14.2	..
4.6	13.2	..	13.3	..	15.0
4.5	11.4	13.3	..	13.3	..
4.4	11.1	..	13.6	..	..
4.3	10.8	12.0	..	..	..
4.0	11.3	12.0	12.7	11.7	11.4
3.7	13.6	16.3	12.4	..	..
3.6	13.0	..	..	..	11.4
3.5	15.5	..	..	13.7	..
3.3	..	37.0	13.9	..	15.4
3.1	17.2	69.4	..	..	..
3.0	..	77.0	16.3	..	..
2.6	..	..	..	75.9	..
2.5	..	..	22.7	..	28.1
2.1	..	..	..	85.9	33.3
2.0	..	..	..	..	21.7
1.7	..	..	46.6	..	..
1.5	..	..	..	..	16.2
1.1	..	..	..	..	11.9
0.8	..	..	..	..	9.2

^aBased on total Kjeldahl nitrogen content of the meal.

^bAverage of several determinations of nitrogen dispersed by distilled water.

TABLE 16

PRECIPITATION OF SOYBEAN PROTEIN FROM ALKALINE DISPERSIONS

pH of Solution	% N ^a Left in Dispersion after Precipitation			
	A, 0.05 N Na ₃ PO ₄ ; B, H ₃ PO ₄	A, 0.05 N Na ₃ PO ₄ ; B, H ₂ SO ₄	A, 0.05 N Na ₂ SO ₃ ; B, H ₂ SO ₄	A, 0.05 N NaOH; B, H ₂ SO ₄
11.4	..	..	..	97.7
10.8	96.5	96.5	..	..
5.7	41.0	..	..	..
5.5	..	45.0	..	..
5.4	..	..	44.9	..
5.0	..	15.5	..	15.3
4.7	12.5	..	..	..
4.6	..	13.4	..	14.1
4.5	12.2	..	..	14.5
4.4	..	12.9	..	..
4.2	..	..	16.2	..
4.1	..	12.9	..	13.4
4.0	12.1	..	15.7	..
3.9	..	..	..	13.8
3.8	..	15.3	..	14.5
3.7	14.7	..	17.2	..
3.6	23.3	..	..	..
3.5	..	..	..	14.6
3.4	..	..	21.0	..
3.1	..	30.9	..	..
2.8	88.7	..	..	..
2.7	..	..	..	21.1
2.5	..	..	48.2	..
2.3	..	65.1	..	..
2.0	..	53.2	..	..
1.9	96.0	..	..	..
1.3	..	74.2	..	..
0.9	..	59.1	..	..

^aBased on total Kjeldahl nitrogen content of the meal; A = extracting agent, B = precipitating agent.

In order to compare the qualitative behavior of other vegetable proteins with that of soybean protein, similar electro-dialyses were conducted with aqueous protein dispersions prepared from the oil-free Alaska pea and rye meals used in Part I. The pH of the pea dispersion, which contained about 80% of the total nitrogen of the meal, was initially 6.5; it dropped to 3.2 in 8.5 hours. A noticeable precipitate appeared at a pH of about 5.2 and was still present at the final pH of 3.2. No further change in pH occurred during an additional 11 hours of electrodialysis.

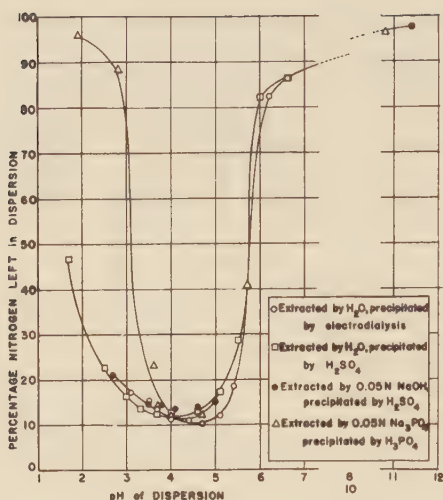


Fig. 6.--Precipitation of soybean protein from water and alkaline dispersions.

In the case of the rye dispersion, which contained only about 40% of the total nitrogen of the rye meal, the pH dropped from 6.6 to 3.0 during 14 hours of electrodialysis, but no precipitation took place.

Preparation of Protein.--After the protein was precipitated at a pH of 4.1 from an aqueous or alkaline dispersion by acid or electrodialysis, it was washed twice with water, centrifuged after each washing, and dried in a forced draft at room temperature.

The actual yields of proteins from 50 grams of meal for several pairs of dispersing and precipitating agents are given in Table 17 in terms of the percentage of total nitrogen in the meal that was recovered in the form of dry protein (column 6). The differences between the values in column 3 (percentage of total nitrogen extracted from the meal) and those in column 4 (percentage of total nitrogen left in dispersion after precipitation of the protein) are reported in column 5. These values are a measure of the maximum amounts of protein that might be recovered by the various processes listed in the first two columns if no losses occurred during their isolation.

TABLE 17

SUMMARY OF DATA ON THE EXTRACTION AND PRECIPITATION OF
SOYBEAN PROTEIN SHOWING ACTUAL PROTEIN YIELDS BASED
ON TOTAL NITROGEN IN THE OIL-FREE MEAL^a

	1	2	3	4	5	6	7	8	9
	Extg. Soln.	Pptg. Agent	% N Ext'd.	% N Left in Disper- sion	% N Re- moved from Disper- sion	% N Actual Protein Yield	Analysis of Pro- teins Recovered		
							Percentages		
							H ₂ O	N	Ash
A	0.05 N NaOH	H ₂ SO ₄	97.7	13.4	84.3	84	8.05	13.49	1.24
B	0.05 N NaOH ^b	H ₂ SO ₄	97.7	12.9	84.8	81	7.53	13.57	1.41
C	0.15 N NaOH ^b	H ₂ SO ₄	97.7	24.9	72.8	67	7.43	13.30	..
D	0.05 N Na ₃ PO ₄	H ₃ PO ₄	96.5	12.1	84.4	81	8.65	13.44	2.87
E	H ₂ O	Electro- dialysis	86.2	11.0	75.2	71	9.05	14.20	..
F	H ₂ O	H ₂ SO ₄	86.2	12.7	73.5	65	7.15	14.48	0.48
G	HCl	NaOH	84.5	..	..	63	10.10	13.93	..

^aPrecipitation at pH 4.1.

^bHydrolyzed at room temperature for 24 hours.

The data in rows A, B, and C illustrate the possible hydrolytic action of sodium hydroxide during the extraction procedure. The data in A and B refer to extraction by 0.05 N sodium hydroxide and precipitation by addition of sulfuric acid to pH 4.1, but the alkaline extract in example B was allowed to stand at room temperature for 24 hours, whereas the protein in A was precipitated almost immediately after extraction. It may be concluded from these data that 0.05 N sodium hydroxide does not hydrolyze the protein appreciably. In example C, however, where the extract with 0.15 N sodium hydroxide was allowed to stand at room temperature for 24 hours, the 12% increase in soluble nitrogen is evidence that hydrolysis took place.

Example A indicates that 13.4% of the total nitrogen is left in dispersion after the acid precipitation. A series of ex-

periments using various protein precipitants was performed to determine the character of the remaining nitrogen and the feasibility of increasing the yield of commercially useful protein. These protein precipitants were added in suitable concentrations to portions of the centrifugate (pH 4.1) from the sulfuric acid precipitation in example A. Aluminum sulfate, chromium sulfate, picric acid, and formaldehyde had no effect. Basic lead acetate decreased the percentage of total nitrogen left in dispersion from 13.4 to 8.7%, a drop of 4.7%. Trichloroacetic acid (final concentration 1 N) decreased the percentage of total nitrogen left in dispersion by 5.4%, phosphotungstic acid by 7.3%, and tannic acid by 8.0%. Thus the total amount of protein, in terms of percentage of total nitrogen in the meal, precipitable from a 0.05 N sodium hydroxide extract by sulfuric acid (84.3%), followed by tannic acid (8.0%), is 92.3%.

Discussion

Examination of the data in Tables 15 and 16 and the precipitation curves in Fig. 6 for the various acid reagents indicates that the maximum precipitation of protein occurs at pH 4.0 to 4.2. The general form of these precipitation curves is similar to that of the nitrogen extraction curves reported in Part I, Fig. 3, with the minimum point occurring in the same pH range.

The curve for precipitation by electrodialysis is similar to those for acid precipitation but does not cover as wide a range of pH values. The protein begins to precipitate at approximately pH 5.2, reaches its maximum precipitation in the pH range 4.3 to 4.7, and then begins to redissolve as the pH becomes lower. At the minimum point on the curve the percentage of nitrogen left in dispersion is about 11%, which is a little lower than the average minimum for the acid precipitation curves. The minimum on the electrodialysis precipitation curve occurs also at a slightly higher pH value than does the average minimum for the other curves. These differences may be caused by a partial removal of the salts during electrodialysis.

The formation of acid in the middle compartment of a three-compartment dialyser has been noted by several workers.³⁹⁻⁴⁵

⁴⁵Oka, J. Soc. Chem. Ind., Japan, 34, Suppl. Binding, 4 (1931).

Bradfield and Bradfield³⁹ found that the amount of acid formed was governed by the type of salt being electrodialed and the character of the membrane. According to their work and also that of Oka,⁴⁵ who studied the electrodialysis of potassium chloride solutions through collodion and parchment membranes, the electrokinetic or zeta potential of the diaphragm used in the electro-dialyzer alters the mobility of the ions through the membrane, leading to a disturbance of neutrality. Parchment paper is charged negatively with respect to a potassium chloride solution; it decreases the transport number of anions and increases that of cations. With negative diaphragms on both the anode and cathode compartments of a three-compartment cell, the cations are removed more rapidly than the anions, and the solution in the middle compartment becomes more acid. Thus it is evident that, in the procedure followed, there is no significant difference between precipitation of the protein by acids and by electrodialysis. However, one would expect from the work of Ettisch and de Loureiro⁴⁰ that continued electrodialysis would eventually remove all of the diffusible salts and the pH of the solution would approach the isoelectric point of the protein.

Unless a procedure can be devised that will maintain a constant pH in the middle compartment while the electrolytes are being removed, it appears that electrodialysis offers no advantages over acid precipitation in the preparation of soybean protein. The Bradfields³⁹ found that the least disturbance of pH resulted from using a parchment-paper cathode membrane and an anode membrane of collodion coated with hemoglobin. Likewise, Ettisch and de Loureiro⁴⁰ claim that a membrane of collodion containing glycine is permeable to anions. Using such a membrane at the anode and parchment paper at the cathode, they were able to avoid marked changes in the middle compartment on electrodialyzing blood serum. Komogata and Shinohara⁴⁶ report that porous aluminum oxide plates furnish a suitable positive diaphragm for electrodialysis. It may be that the use of a positive type diaphragm, such as alundum, on the anode compartment would permit the removal of salts from soybean protein without the disturbing influence of acid formation; however, since the types of salts present are a

⁴⁶Komogata and Shinohara, J. Inst. Elec. Engrs. Japan, 51, 115 (1931).

factor, a solution of the problem may not be found so easily.

From observations made on the character of the several proteins listed in Table 17, during extraction, precipitation, and drying, it appears that the extracting agent exerts greater influence on the properties of the protein than does the precipitating agent. The protein extracted with water and precipitated with sulfuric acid occupied, in the wet state, about half the volume occupied by proteins extracted with alkaline solutions and precipitated by the same acid. The water-extracted protein was composed of bigger and heavier flocs; it settled more rapidly and dried much faster than the others, which indicated that it was not hydrated so much. In the dry state it was more friable and had a lighter color.

The color of the soybean proteins prepared by the methods described is yellow-brown. This is a serious drawback in their industrial utilization, especially in the fields of plastics and paper coatings. If the protein is dried by the use of alcohol and ether^{10,12} or over sulfuric acid in a refrigerator under vacuum, a much lighter colored product (light gray) is obtained; but it is a false whiteness, probably caused by the physical state of subdivision, and the yellow-brown color returns when the protein is again dispersed in alkali or pressed into a plastic mixture. The production of a white or nearly white soybean protein appears to be a difficult problem, but if solved it will add greatly to the industrial usefulness of this protein.

The protein-tannic acid complex that is isolated from the centrifugate following the sulfuric acid precipitation represents an additional 8.0% of the total nitrogen in the meal. Results of preliminary experiments indicate that this complex may be usable in certain of the protein-type plastics and, if substantiated, presage a more efficient utilization of the nitrogen-containing constituents of the soybean. For reasons of economy the crude vegetable tanning liquors similar to those used in the leather industry would probably be substituted for the tannic acid.

The various procedures discussed above show that 92.3% of the total nitrogen in oil-free soybean meal is accounted for by the sulfuric acid and tannic acid precipitates from alkaline extracts. This nitrogen may be assumed to represent protein. Thus, almost all of the protein in oil-free soybean meal is readily available. In establishing commercial procedures for its sepa-

ration, high yields may be anticipated.

Summary

Soybean protein was extracted from oil-free meal with water, 0.05 N sodium hydroxide, and 0.05 N Na_3PO_4 , and precipitated by sulfuric or phosphoric acid at various pH values. The protein in aqueous dispersion was also precipitated by electro-dialysis through parchment paper. In addition, one protein sample was extracted from the meal with 0.07 N hydrochloric acid and precipitated by sodium hydroxide. The acids produced maximum precipitation at pH 4.0 to 4.2, the electrodialysis at pH 4.3 to 4.7.

Extraction with 0.05 N sodium hydroxide and precipitation with sulfuric acid yielded the most protein. Eighty-four per cent of the nitrogen in the meal was recovered as protein in this way.

An additional 7.3% of the total nitrogen could be precipitated by phosphotungstic acid from the centrifugate after sulfuric acid precipitation, and 8.0% by tannic acid.

In the precipitation of soybean protein there were no marked differences between the action of acids and of electro-dialysis through parchment paper.

PART III

THE EFFECT OF FORMALDEHYDE ON THE ISOELECTRIC POINTS OF SOYBEAN AND OTHER PROTEINS BY MICROELECTROPHORESIS

Introduction

The action of formaldehyde on proteins is of practical as well as theoretical interest. Formaldehyde has long been used industrially to increase the water resistance of proteins, especially casein, in the plastics and coatings fields. Formaldehyde is also extensively employed as a preservative and fixative for biological specimens and to block off the amino groups in the titration of the acid groups of proteins and amino acids.

An appreciable literature has accumulated concerning the nature of the reaction of formaldehyde with proteins and amino acids. Since reviews on this subject are available,⁴⁷⁻⁴⁹ it will not be discussed in detail. All the theories possess in common the postulate that the formaldehyde reacts with the free basic (amino, guanidino, or imino) groups of the protein or amino acid, but differ in the complexities of the structures proposed for the product.

According to the postulated reactions, the free basic groups on the micelle or molecule are rendered relatively inactive by the action of formaldehyde. This should lessen the positive charge on the micelle and result in a shift of the isoelectric point to a lower pH value, since at any given pH the protein is less basic than before.

It has been demonstrated by Smith, Max, and Handler⁵⁰ that

⁴⁷Highberger and O'Flaherty, J. Intern. Soc. Leather Trades' Chem., **23**, 549 (1939).

⁴⁸Levy and Silberman, J. Biol. Chem., **118**, 723 (1937).

⁴⁹Theis and Priestly, J. Am. Leather Chem. Assoc., **34**, 566 (1939).

⁵⁰Smith, Max, and Handler, J. Phys. Chem., **43**, 347 (1939).

the points of minimum solubility of casein and soybean protein are shifted to lower pH values by the action of formaldehyde. Gerngross and Bach⁵¹ found that in gelatin solutions containing 10% formaldehyde the isoelectric point of one gelatin sample shifted from a pH of 5.05 to 4.6 and of another from 4.75 to 4.3, as determined by an electrophoretic transference technic. Also, Swyngedauw,⁵² from a study of electroösmosis in gelatin gels before and after formaldehyde treatment, claims that formaldehyde does not combine with gelatin on the acid side of the isoelectric point but does on the basic side. Mudd and Joffe⁵³ studied the modification of antibodies by formaldehyde and showed a shift to lower pH values for their isoelectric points, as well as a decreased tendency to agglutinate.

The method of electrophoresis is the most direct means of determining the isoelectric point of a protein, and the type of cell employing microscopic technic is the most convenient to use. Accordingly, for this study a microelectrophoretic apparatus was used to determine the effect of formaldehyde on the isoelectric points of solvent-extracted oil-free soybean meal, of several soybean protein samples prepared by different methods, of a commercial soybean protein, and of casein, gelatin, and egg albumin, which were included for comparison. These data should be useful in the industrial application of these proteins. It is hoped further that eventually the number of proteins studied will be increased, so that the shift in their isoelectric points when treated with formaldehyde may be correlated quantitatively with what is known concerning their composition and structure.

Experimental

Apparatus and Methods.--The apparatus and methods used were modified somewhat from those suggested by Abramson, Moyer, and others.⁵⁴⁻⁵⁶ Two Abramson type horizontal microelectrophoresis

⁵¹Gerngross and Bach, Biochem. Z., 143, 533 (1923).

⁵²Swyngedauw, Compt. rend. soc. biol., 130, 468 (1939).

⁵³Mudd and Joffe, J. Gen. Physiol., 16, 947 (1933).

⁵⁴H. A. Abramson, "Electrokinetic Phenomena," The Chemical Catalog Co., Inc., New York, 1934.

⁵⁵Moyer, J. Bact., 31, 531 (1936).

cells were used, one in which the ratio of width to depth was 28 and the other, 14. This all-glass cell, which is described in detail by Moyer,⁵⁵ has a central compartment in the form of a rectangular parallelepiped, sealed at both ends to four-way stop-cocks with right-angle bores. The electrode compartments are separated from the central part by plaster of paris plugs saturated with sodium sulfate, and contain non-polarizable copper-copper sulfate (saturated) electrodes.

In the central compartment, internal dimensions of which may be 0.5 mm. x 11 mm. x 40 mm., and which may have optical flats on top and bottom 0.5 mm. thick, the flow of solution under the influence of a direct current is completely laminar. Since the cell is a closed one, the electroosmotic flow along the walls must return at the center, which means that at some point between the wall and center there is no electroosmotic flow. The theory of this cell is discussed in detail by Abramson.⁵⁴

Microscopic observations were made in the two cells at the stationary electroosmotic levels, 0.21 and 0.79 of the cell depth in the first cell, and 0.20 and 0.80 of the cell depth in the second. A majority of determinations were made in the first cell, several of which were checked in the second. The microscopic equipment included a 20x ocular containing a field micrometer disk divided into 0.5 mm. squares, a 10x objective, and a 21x objective.

The cell in use was standardized periodically by measuring the mobility of human erythrocytes in M/15 phosphate buffer at pH 7.4, as suggested by Abramson.⁵⁷ The experimental values were within 2% of the standard reference value of 1.31×10^{-4} cm.² volt⁻¹ sec.⁻¹ at 25° C. The mobilities of the proteins studied were determined from the observation of quartz particles of microscopic size in the protein solutions. It has been shown that inert particles, such as quartz, placed in a protein dispersion, become coated with a film of protein and acquire the mobility of the protein itself.^{54,58} In almost all cases this mobility agrees

⁵⁶Sookne and Harris, J. Research Natl. Bur. Standards, 23, 299 (1939).

⁵⁷Abramson, J. Gen. Physiol., 12, 711 (1929).

⁵⁸Moyer, Cold Spring Harbor Symposia Quant. Biol., 6, 228 (1938).

with that of the dissolved protein as determined by electrophoresis in a U-tube apparatus, such as that of Tiselius.⁵⁸

Thirty minutes or more were allowed for the cell to come to equilibrium after filling with a protein dispersion containing suspended microscopic particles of quartz. All measurements were carried out at room temperature, which varied from 24°-29° C., and were corrected to 25° C. by applying a factor of 2% per degree.^{54,55} At least 10 measurements of transit time were made using a double action 0.1 second stopwatch and timing alternately in both directions.

The formula, $u = \frac{d}{t} \frac{q}{I} \frac{K}{R}$ cm.² volt⁻¹ sec.⁻¹, was used in calculating the mobility u , where d is the distance of transit of the particle observed in time t , q is the cross section of the cell at the point of observation, I is the current passing through the cell, and R is the resistance of the protein dispersion as determined in a conductivity cell with constant K . In case the values of t in opposite directions did not agree closely, the reciprocals of t were averaged rather than the values themselves, which is the same as averaging the velocities in opposite directions.

The experimental technic requires that many precautions be observed. The microelectrophoresis cell should be firmly fastened in place on the stage of the microscope (in the present instance, it was cemented to a brass holder attached to a mechanical stage). Both the cell and microscope should be level, with the optical axis of the microscope perpendicular to the plane of the optical flats. The cell depth is best determined by allowing quartz particles to settle on the top and bottom internal faces of the optical flats, and focusing upon them with a graduated fine adjustment. In practice the fine adjustment is left in focus on the calculated stationary electroosmotic level (in this instance, the upper level, although data obtained at the lower level are used as a check). Observations are made only on particles which are in focus at this level. If the particle under observation should settle out of the field of view, another should be chosen which is in focus. Leaks around the stopcocks due to faulty greasing may lead to streaming of the fluid even when the current is shut off, in which case the velocities of the particle in opposite directions (obtained by reversing the current) will be unequal. Air bubbles should be excluded from the central and

electrode compartments, as they also may contribute to anomalous streaming. Dilute chromic acid cleaning solution was found excellent for cleaning the cell, including the removal of protein adsorbed on the walls, but should not be allowed to come in contact with the plaster of paris plugs. The cell should be thoroughly rinsed with distilled water after cleaning. Other details of technic are given by Moyer.⁵⁵

The pH measurements were carried out on the same portable glass electrode pH meter used previously; they were referred to 0.05 M potassium acid phthalate as a standard, to which was assigned pH 4.01.⁵⁹

Materials.--The various soybean protein samples (except for the commercial soybean "Alpha" protein) were prepared from 1938 Illini soybeans, which were cracked, flaked, extracted with cold petroleum ether as in Part I, and air-dried at room temperature. Details on the separation of soybean protein from the oil-free meal will be found in Part II. Briefly, the proteins used in this study were prepared and designated as follows:

I. Oil-free soybean meal, prepared as above and ground to pass a 100-mesh screen.

II. Electrodialyzed soybean protein, extracted from oil-free meal with water and precipitated by electrodialysis through parchment paper from initial pH 6.6 to pH 3.1.

III. Electrodialyzed soybean "whey," the solution left from the electrodialyzed soybean protein II after the precipitated curd was removed by centrifuging.

IV. Acid-precipitated soybean protein, extracted from oil-free meal with water and precipitated with sulfuric acid at pH 4.1.

V. Reprecipitated soybean protein, extracted from oil-free meal with water, precipitated with sulfuric acid at pH 4.1, redispersed in sodium hydroxide at pH 8.0, precipitated with sulfuric acid, and the last two steps repeated.

VI. Alkali-treated soybean protein, extracted from oil-free meal with water, precipitated by sulfuric acid, redispersed in 0.4 N sodium hydroxide, allowed to stand at room temperature for 20 hours, and precipitated with sulfuric acid at pH 4.4.

VII. Salt-extracted soybean protein,¹⁰ extracted from oil-

⁵⁹Hitchcock and Taylor, J. Am. Chem. Soc., 60, 2710 (1938).

free meal with 10% sodium chloride, clarified by supercentrifugation rather than by filtration, precipitated by dialysis through viscose casing, and dried with alcohol and ether.

VIII. "Alpha" protein, a commercial soybean protein.

IX. Casein, precipitated from fresh skim milk initially at pH 6.6 by electrodialysis in a three-compartment dialyzer with parchment membranes at a final pH value of 3.6.

X. Electrodialyzed egg white solution, obtained from fresh sterile eggs by diluting the egg whites with water, stirring to break the membranes, filtering and electrodialyzing through parchment paper, filtering again, and storing in the refrigerator until used.

XI. Crystalline egg albumin, prepared with sodium sulfate by the method of Kekwick and Cannan.⁶⁰

XII. Gelatin, Coignet gold label brand.

Protein samples II, IV, V, VI, and IX were centrifuged, washed several times with water, and stored as wet curd in the refrigerator until use which occurred as soon after preparation as feasible.

The formaldehyde was c.p. grade, 40% by volume. The standard sodium acetate was prepared stoichiometrically from standard sodium hydroxide and acetic acid.

The proteins were adsorbed for observational purposes on quartz particles prepared by grinding in a ball mill. The ground quartz was levigated in distilled water, cleaned by boiling in a mixture of concentrated nitric and hydrochloric acids, rinsed about 20 times with distilled water, and dried. A centrifuge was used between rinses for sedimentation of the particles, which were 0.5 to 5 microns in diameter. Before using, the particles were suspended in distilled water.

Procedure.--Mobility measurements on casein and soybean protein were conducted in the following way. Fifty g. of wet curd (10 g. in case of the dry "Alpha" protein and salt-extracted soybean protein) was dissolved by trituration with dilute sodium hydroxide and sodium acetate, and diluted to 1000 ml. so that the final solution was 0.02 N in sodium hydroxide and 0.02 N in sodium acetate. This was immediately divided into 50-ml. aliquots placed in centrifuge bottles, to which were added in duplicate varying

⁶⁰Kekwick and Cannan, Biochem. J., 30, 227 (1936).

amounts of acetic acid to reach the desired pH values. To half of the samples was added 50 ml. of 40% formaldehyde and to the other half 50 ml. of water, after which they were shaken 30 minutes, centrifuged, poured into 200-ml. volumetric flasks containing 10 ml. of quartz suspension, and diluted to the mark. The final solutions were thus approximately all the same in ionic strength, $\mu = 0.01$, with half of them being 10% by volume in formaldehyde. The pH values ranged from 6.5 to 3.7. The cell was rinsed several times with the protein-coated quartz suspension of highest pH value, allowed to stand 30 minutes, and the mobility determined. The protein-formaldehyde quartz suspensions were treated in the same manner. This procedure was used for proteins II, IV, V, VI, VII, VIII, and IX.

The isoelectric point of soybean meal was determined by mobility measurements on the aqueous extract which was prepared by extracting 25 g. of oil-free meal with 800 ml. of water, clarifying by centrifuging, diluting to 1000 ml. with enough sodium acetate solution to make it 0.04 N in sodium acetate, dividing into aliquots, and treating as in the above-described procedure. Although the final solutions of the aqueous extract were 0.01 N in sodium acetate, it must be emphasized that the ionic strength was higher owing to the aqueous extractants other than protein which are present. Besides salts, the aqueous extract contains carbohydrates, phosphatides, and other substances.

In the case of the electrodialyzed soybean "whey" enough sodium acetate was added to make the final solutions 0.01 N in sodium acetate. Since no precipitate appeared on adding acetic acid, the centrifugation was omitted in the procedure.

Mobility measurements on the gelatin and egg albumin samples were carried out similarly, but since both egg albumin and gelatin are water soluble and not precipitated by acids, no alkali or centrifugation was required. The crystalline egg albumin was dissolved in water and dialyzed through viscose casing. The final solutions of both the crystalline egg albumin and electrodialyzed egg white were approximately 0.5% in protein concentration and 0.01 N in sodium acetate. The gelatin solutions contained 0.2% protein and 0.02 N sodium acetate.

The usual concentration of formaldehyde in the protein solutions was 10% by volume. In addition, the effect of concentration of formaldehyde was determined by treating the gelatin

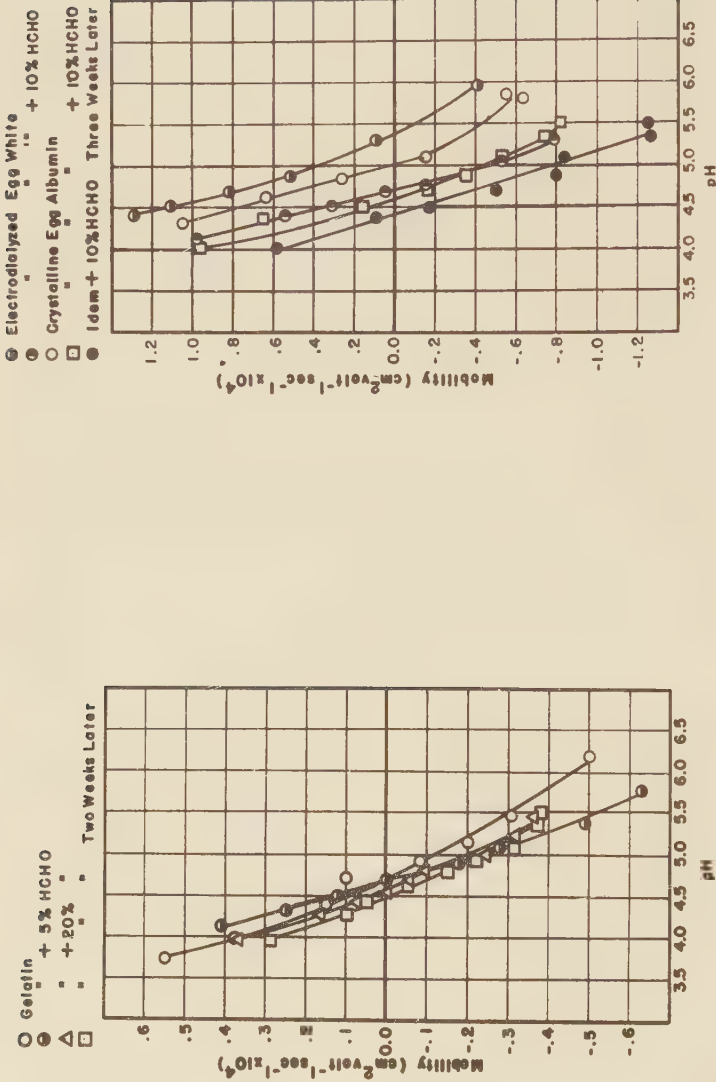
with 5% and 20% formaldehyde, the aqueous soybean meal extract with 5% formaldehyde, and the acid-precipitated soybean protein with 1%, 5%, and 20% formaldehyde. The mobility determinations were carried out within 2 to 4 hours of preparation of the protein-formaldehyde solutions. A further study of the effect of time on the reaction between protein and formaldehyde was made on certain of the samples by allowing them to stand at room temperature and redetermining their mobilities 1 to 3 weeks later.

Results and Discussion

Gelatin.--The mobility data for gelatin with and without formaldehyde are plotted in Fig. 7. This particular gelatin sample appears to be little affected by formaldehyde, as 5% concentration of formaldehyde did not shift the isoelectric point at all, and 10% and 20% lowered it by only 0.1 of a pH unit. These results do not agree well with those of Gerngross and Bach,⁵¹ who reported a lowering in the isoelectric point of 0.45 pH unit for each of two different gelatin samples following 10% formaldehyde treatment. Lacking other evidence, the quantitative discrepancy between their results and those presented here may be attributed to differences in the gelatin samples.

Fig. 7 is an indication also of the effect of both concentration of formaldehyde and the time of reaction on the gelatin mobility-pH curves. The effect of an increase from 5% to 20% in the concentration of formaldehyde is to shift the isoelectric point of gelatin from pH 4.7 to pH 4.6. The data for the 20% formaldehyde-gelatin solutions were redetermined 2 weeks later on the same samples. The pH values of these buffered solutions did not change significantly in the period of 2 weeks. Attention should be directed, however, to the change in mobilities and especially to the shift of the isoelectric point to pH 4.5, a lowering of 0.1 pH unit with time. Thus, concentration of formaldehyde and reaction time are both small but positive factors in the shift of the isoelectric point of gelatin to lower pH values.

Egg Albumin.--The effect of formaldehyde on both the crystalline egg albumin and the electrodialyzed egg white is quite marked, as shown in Fig. 8. The isoelectric point of the latter is shifted from pH 5.4 to pH 4.7 by the action of 10% formaldehyde, and that of the former from pH 5.0 to pH 4.6. It would ap-



pear that inadvertently the crystalline egg albumin had become surface-denatured, as its isoelectric point checks with that of Moyer⁶¹ for the surface-denatured egg albumin, but not with the value for the crystalline egg albumin, pH 4.82⁶¹ or pH 4.86.⁶² The effect of time of reaction on the mobilities of the formaldehyde-crystalline egg albumin solutions is also shown in Fig. 8. The pH values of the buffered solutions did not change over a period of 3 weeks, but the mobilities did, and the isoelectric point shifted to a pH value of 4.4, a lowering of 0.2 pH unit with time.

Casein.--Moyer⁶³ determined the isoelectric point of casein prepared by the method of Van Slyke and Baker to be pH 4.51. In the range of low casein solubility, i.e., in the region of the isoelectric point, he made direct observations on flocculated casein particles rather than on inert quartz particles coated with casein, as is done here. Considering the differences in preparation and technic, the value, pH 4.6, as shown in Fig. 9 checks well with his. The mobility-pH curve for the formaldehyde-casein dispersion reveals a shift of the isoelectric point to pH 4.2, a lowering of 0.4 of a pH unit owing to the action of the formaldehyde.

Soybean Protein Preparations.--Fig. 10 shows the effect of formaldehyde on the isoelectric points of oil-free meal and "Alpha" protein. The isoelectric point of the meal was found to be at a pH value of 4.1, which agrees with that of approximately 4.1 determined by Monaghan-Watts⁶⁴ in a Mattson cylindrical type microelectrophoresis cell, and also with the value of approximately 4.1 found in Parts I and II as the minimum in the extractability (Fig. 3) and precipitability (Fig. 6) of the nitrogenous matter of the meal. Monaghan-Watts observed meal particles suspended in 0.02 M acetate buffers, whereas in this instance observations were made on quartz particles suspended in aqueous meal extracts 0.01 N in sodium acetate which had been clarified by centrifuging.

The action of 5% and 10% formaldehyde on the meal did not

⁶¹Moyer, J. Phys. Chem., **42**, 71 (1938).

⁶²Smith, J. Biol. Chem., **113**, 473 (1936).

⁶³Moyer, J. Biol. Chem., **133**, 29 (1940).

⁶⁴Monaghan-Watts, Ind. Eng. Chem., **29**, 1009 (1937).

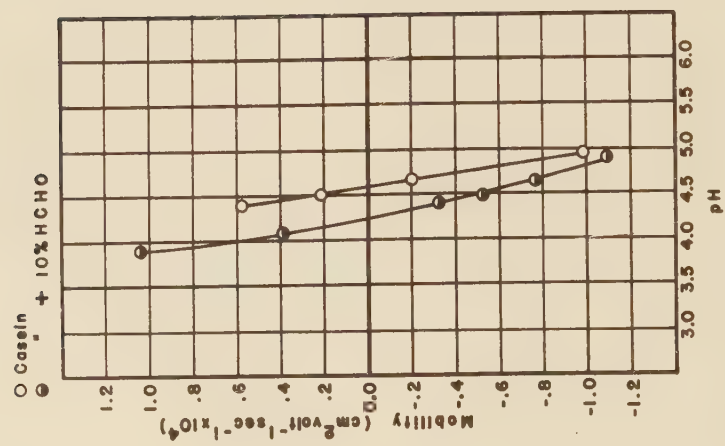


Fig. 9.--Mobility-pH curves for electrodialyzed casein in acetate buffers of ionic strength 0.01, with and without formaldehyde.

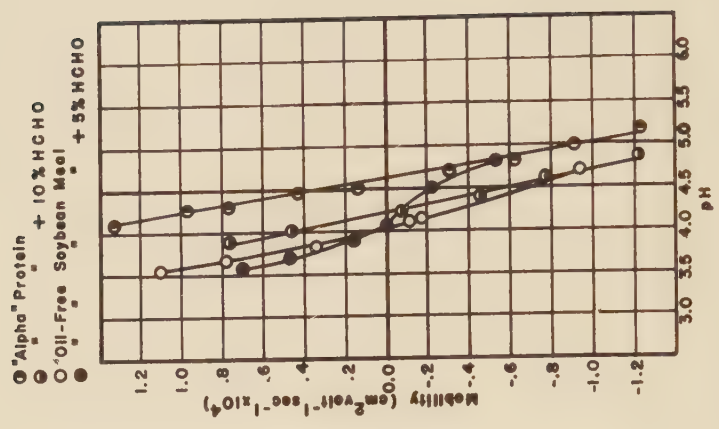


Fig. 10.--Mobility-pH curves for commercial soybean "Alpha" protein and oil-free soybean meal, in acetate buffers of approximately 0.01 ionic strength, with and without formaldehyde.

alter its isoelectric point. This might be expected from a previous study by Smith, Max, and Handler,⁵⁰ in which it was shown that the pH of minimum solubility of the nitrogenous constituents of oil-free soybean meal was not appreciably altered by the presence of 10% formaldehyde in the dispersions. The absence of any effect on the isoelectric point of the meal is especially interesting as all purified samples of soybean protein showed a marked change in isoelectric point following treatment with formaldehyde. This should not be construed necessarily to mean that the extractants in an aqueous extract of soybean meal do not react with formaldehyde, but merely that such a reaction is not indicated by the criterion of a shift in isoelectric point following such treatment. That some type of reaction has occurred is shown by the marked difference in the shapes of the mobility curves for the meal with and without formaldehyde, despite the congruence of isoelectric points. Fig. 10 also shows that 10% formaldehyde shifted the isoelectric point of "Alpha" protein from pH 4.6 to pH 4.2.

The data for electrodialyzed soybean protein, electrodialyzed soybean "whey," and acid-precipitated soybean protein are plotted in Fig. 11. The near congruence of isoelectric points for the acid-precipitated and electrodialyzed proteins confirms the conclusion reached in Part II that the proteins prepared in the manner indicated do not differ greatly in their properties. The isoelectric point of the electrodialyzed soybean protein was shifted by the action of 10% formaldehyde from a pH value of 4.2 to 3.9, while that for the electrodialyzed soybean "whey" shifted from a pH of 5.1 to 4.4.

Becker, Milner, and Nagel²⁵ have shown that the "whey" contains approximately 11% of the total nitrogen of the meal and that about 5% of the total nitrogen is dialyzable through cellophane. This leaves 6% of the total meal nitrogen which is precipitable by tannic acid (Part II) and which appears to behave as a protein with an isoelectric point of 5.1.

Table 18 shows the effect of time and concentration of formaldehyde on the isoelectric point of acid-precipitated soybean protein. This checks the conclusion reached with gelatin that both the concentration of formaldehyde and the time of reaction are positive factors in shifting the isoelectric point of the protein to lower pH values.

In Fig. 12 are plotted mobility-pH curves for alkali-

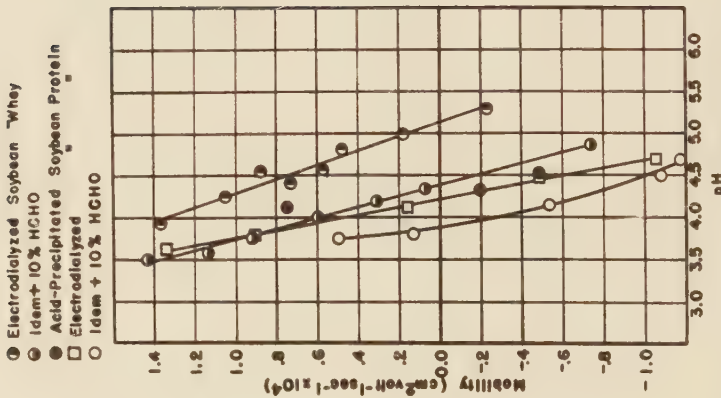


Fig. 11.--Mobility-pH curves for electrolyzed soybean protein and electrolyzed soybean whey, with and without formaldehyde, and acid-precipitated soybean protein, in acetate buffers of ionic strength 0.01.

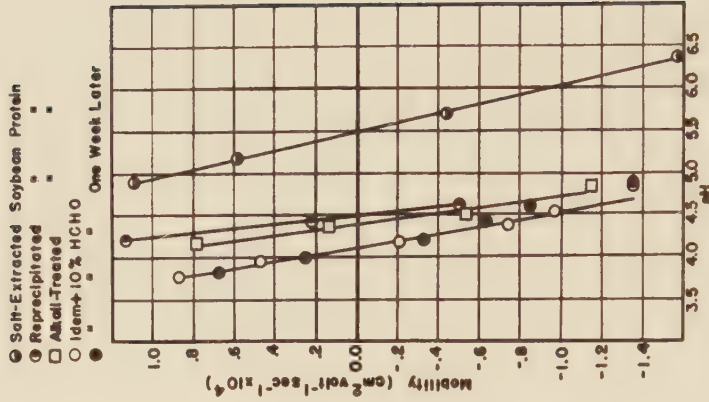


Fig. 12.--Mobility-pH curves for alkali-treated soybean protein, with and without formaldehyde, reprecipitated soybean protein, and salt-extracted soybean protein, in acetate buffers of ionic strength 0.01.

TABLE 18

EFFECT OF TIME AND CONCENTRATION OF FORMALDEHYDE ON THE
ISOELECTRIC POINT OF ACID-PRECIPITATED SOYBEAN PROTEIN^a

Concentration of HCHO %	IP ^b of HCHO-Treated Protein (2-4 hours' duration) pH	IP of HCHO-Treated Protein (9 or more days' duration) pH
1	4.1	3.8
5	3.9	3.7
10	3.8	3.6
20	3.8	3.7

^aIP of untreated protein = 4.3.

^bIP = isoelectric point.

treated soybean protein, reprecipitated soybean protein, and salt-extracted soybean protein. The alkali-treated soybean protein-formaldehyde curves are also shown, both for the freshly-prepared dispersions and the same 1 week later. Formaldehyde shifted the isoelectric point of the alkali-treated soybean protein from a pH value of 4.4 to 4.1, which latter value was unaltered 1 week later, even though the mobilities of the buffered solutions had changed somewhat. It is interesting to note that the isoelectric point of the reprecipitated soybean protein, pH 4.5, is slightly higher than that of the alkali-treated, showing that this property of the acid-precipitated soybean protein is more markedly altered by several alternate treatments with weak alkali and acid than by one prolonged treatment in relatively strong alkali.

The isoelectric point of salt-extracted soybean protein in sodium acetate buffers of ionic strength 0.01, as plotted in Fig. 12, is at a pH of 5.5. This is the highest pH value for the isoelectric point of a soybean protein preparation that has been reported. Csonka, Murphy, and Jones⁶⁵ assigned a pH of 4.7 for the isoelectric point of purified soybean protein, while Hartman and Cheng⁶⁶ determined the isoelectric point of salt-extracted

⁶⁵Csonka, Murphy, and Jones, J. Am. Chem. Soc., 48, 763 (1926).

⁶⁶Hartman and Cheng, J. Phys. Chem., 40, 453 (1936).

soybean protein in potassium acid phthalate-sodium hydroxide buffers of ionic strengths greater than 0.1 to be at a pH value of 5.0 by a transference method. It has been shown for degummed silk⁶⁷ and for egg albumin⁶² in uni-univalent electrolytes that the pH of the isoelectric point is decreased by an increase in ionic strength, so the value for the isoelectric point of salt-extracted soybean presented here is considered to be in fair agreement with that of Hartman and Cheng.

TABLE 19

ISOELECTRIC POINTS OF PROTEINS INVESTIGATED AND SHIFT CAUSED BY FORMALDEHYDE TREATMENT OF 2-4 HOURS' DURATION

Protein	IP ^a of Protein pH	IP of HCHO ^b Treated Protein pH	Shift in IP pH Unit
I. Soybean meal, oil-free (5 and 10 per cent HCHO treatment)	4.1	4.1	0.0
II. Soybean protein, electrodialyzed	4.2	3.9	-0.3
III. Soybean "whey," electrodialyzed	5.1	4.4	-0.7
IV. Soybean protein, acid-precipitated	4.3	3.8	-0.5
V. Soybean protein, reprecipitated	4.5	..	..
VI. Soybean protein, alkali-treated	4.4	4.1	-0.3
VII. Soybean protein, salt-extracted	5.5	..	..
VIII. "Alpha" protein (commercial soybean protein)	4.6	4.2	-0.4
IX. Casein, electrodialyzed	4.6	4.2	-0.4
X. Egg white, electrodialyzed	5.4	4.7	-0.7
XI. Egg albumin, Na ₂ SO ₄ -purified	5.0	4.6	-0.4
XII. Gelatin, Coignet gold label (5 per cent HCHO treatment)	4.7	..	..
(10 and 20 per cent HCHO treatment)	..	4.7 4.6	0.0 -0.1

^aIP = isoelectric point.

^bTen per cent concentration unless otherwise indicated.

It is difficult to explain why salt-extracted soybean protein prepared by the Hartman-Cheng procedure¹⁰ should have an iso-

⁶⁷Sookne and Harris, J. Research Natl. Bur. Standards, 23, 471 (1939).

electric pH so much higher than that for any other soybean protein preparation. It was noted that the buffered salt-extracted protein solutions had a pronounced odor of hydrogen sulfide, which indicates that extraction of the meal with 10% sodium chloride and the subsequent treatment has altered the protein in some manner. The wide spread in the isoelectric points of the various soybean protein preparations studied would indicate that further investigation of the effect of various treatments on their properties is merited.

Table 19 summarizes the isoelectric points of the various protein preparations with and without formaldehyde treatment.

Summary

A microelectrophoretic technic was used to determine the effect of formaldehyde on the isoelectric points of solvent-extracted soybean meal, of several soybean protein samples prepared by different methods, a commercial soybean protein, casein, gelatin, and egg albumin.

Observations were made on protein-coated microscopic quartz particles suspended in aqueous acetate buffers of ionic strength 0.01, and the pH of the isoelectric point of the protein obtained from a mobility-pH graph.

The isoelectric points of all the proteins studied were lowered from 0.1 to 0.7 pH unit by the action of formaldehyde, except for soybean meal, which was unaffected by 5% or 10% formaldehyde.

The concentration of formaldehyde and the time of reaction were small but positive factors in the shift of the isoelectric points to lower values for all but two of the proteins studied.

New data are presented for the isoelectric points of soybean protein samples prepared by several methods.

